STUDIES OF PLANT COMMUNITY STRUCTURE IN RELATION TO HEAVY METAL POLLUTION OF SOIL AT ALIGARH

DISSERTATION

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DEDICATED TO MY PARENTS

"Whose blessings have sustained me through this work"

In the name of Allah, the Beneficent, the Merciful

How can Allah's favours be counted: Look at the earth alone. Life and conditions here are mutually balanced for Allah's creatures. The vegetable world produces fruits of various kinds and corn and grain of various kinds for human food. The grain harvest yield with it fodder for animal in the shape of leaves and straw, as well as food for men in the shape of grain. The plants not only supply food but sweet smelling herbs and flowers.

(Al-Quran: Surah Ar. Rahman, Verse 10-12)

"Plants speak to man but only in whisper; their voice can be heard by those who remain close to them."

(Dr. Norman E. Borlaug)

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Certificate

This is to certify that work embodied in this dissertation entitled "Studies of plant community structure in relation to heavy metal pollution of soil at Aligarh" is a bonafide research work carried out by Mr. Gh. Mohd. Rather under my supervision and is suitable for submission for the M. Phil. degree in Botany of Aligarh Muslim University, Aligarh. No part of this work has been submitted for any other degree or diploma.

Dr. Athar A. Khan
(Research Supervisor)

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Gh. Mohd.

(Gh. Mohd. Rather)

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INTRODUCTION

Environment is an intricate system of various physical, chemical and biological elements that affect each other reciprocally. Like individual elements in any environment, the various environments are closely interlinked. Hence, depending on intensity, the impact of even a local perturbation may be felt at the ecosystem or biosphere level.

Ecosystems are inherently capable of accommodating changes. Indeed, most environments are in a continual state of flux, experiencing ongoing series of adjustments – a state known as dynamic equilibrium. When relative stability can be maintained with only minor adjustments, the environment is said to be in a steady state. Despite this ability to respond to change(s), there are times when the amount of change exceeds the ability of environment to accommodate it. The end result is environmental disruption or environmental pollution.

Even though environmental changes and environmental disruptions occurred regularly in the earth's history, but recent past witnessed an unprecedented and rapid modification of environment, which overrides its natural balancing abilities leading to different environmental problems e.g., climate change and ozone layer depletion etc. The genesis of these and other such environmental problems may be traced to chemical pollution, inorganic as well as organic. The major component of inorganic contaminants is heavy metals (Adriano, 1986; and Alloway, 1990). The heavy metal pollution of

biosphere has accelerated dramatically since the beginning of industrial revolution (Nriogo, 1979). The primary sources of this pollution include the burning of fossil fuels, mining and smelting of metalliferous wastes, fertilizers, pesticides and sewage (Kabata-Pendias and Pendias, 1992). Toxic metal contamination of ground water and soil, which poses a major environmental and human health problem, is currently in need of an effective and affordable technological solution. Moreover, unlike organic pollutants metals can not be degraded to harmless products but, instead, persist indefinitely in the environment, complicating their remediation (Ghosh and Singh, 2005).

HEAVY METALS

Definition

All metals having the specific gravity greater than four (Nieboer and Richardson, 1980), or five or more are included in this category. The term heavy metal includes all the lower members (metals) of the periodic table and is always used in context with environmental pollution. Only those members of periodic table which are (1) relatively abundant in the earth's crust, (2) extracted and used in a reasonable amount, (3) used in places where the public may come in contact with them and (4) toxic to human beings are generally referred to as heavy metals (Martin and Coughtrey, 1982)

Classification

Metals have been classified into three classes Class 'A', Class 'B' and Borderline (Nieboer and Richardson, 1980) depending on their affinity towards different ligands.

Class A:

Metals ions of this class are characterized by small size and low polarizability (sometimes referred to as hard acids). This class includes:

Li, Be, Na, Mg, Al, K, Ca, Fe(III), Rb, Zr etc.

Class B:

The metal ions of this class are characterized by large size and high polarizability (sometimes referred to as soft acids). This class includes:

Cu(I), Pb, Ag, Cd, Au, Hg, Pb(II) etc.

Class borderline:

The metal ions of this class are clearly distinct from those of class A but show increasing degrees of class B characteristics. This class includes:

V, Cr, Mn, Fe(II), Co, Ni, Cu(II), Pb(IV), Sn etc.

The above classification of metals by their Lewis acidity indicates the form of bonding in their complexes. Class A metal ions preferentially form complexes with similar nonpolarizable ligands, particularly oxygen donors and the bonding in these complexes is mainly **ionic**. Class B ions preferentially form complexes with polarizable, soft ligands to give a **covalent** bond formation. Hence the **hard-hard** or **soft-soft** combinations are preferred wherever possible.

Fifty three out of ninety naturally occurring elements are heavy metal. Out of these some are having biological importance. Based on their solubility under physiological conditions seventeen heavy metals may be available for living cells and are of importance for organisms and ecosystem (Weast, 1984). Among these metals Fe, Mo and Mn are important as micronutrients, Zn, Ni, Cu, V, W and Cr are toxic elements with high or low importance as trace elements. As, Hg, Ag, Sb, Cd, Pb and U have no known function as micronutrients and seem to be more or less toxic to plants and microorganisms (Goldbold and Hutterman, 1985; Breckle, 1991; Nies, 1999).

Causes of heavy metal contamination

Metal pollution results when human activity disrupts normal biogeochemical activities. Sometimes a single metal is involved but more often mixtures of metals are present. Mining, ore refinement, industrial manufacturing of batteries, metal alloys, electric components, paints, preservatives and insecticides are examples of processes that produce metal byproducts. Examples of specific metal contaminants include copper and zinc salts that are used extensively as pesticides in agricultural settings, silver salts that are used to treat skin burns, lead which is utilized in the production of batteries, cable sheathing and alloys. The extent of metal pollution becomes even more obvious when one considers the amount of waste generated in metal processing.

While metals are ubiquitous in nature, human activities have caused metals to accumulate in soil. Such contaminated soils provide a metal source

from which surface waters, ground waters etc., can become contaminated. Metal contamination has occurred for centuries since metals have been used extensively throughout human history (Nriagu, 1996).

Heavy metals in lithosphere and hydrosphere

Soil usually exhibit higher concentrations of metals than waters because metals are more likely to accumulate in soil versus being diluted or carried elsewhere in water and soils are composed of minerals which can naturally contain high concentrations of metals. The cation exchange capacity of soils allows metals to attach to soil particles in response to ionic attractions and accumulate.

Soil factors and heavy metal toxicity

Several biotic and abiotic factors can affect the chemical speciation of metals in soil and thus affect the bioavailability and toxicity of metals to soil dwelling organisms. These factors include metal chemistry, sorption to clay minerals and organic matter, pH, redox potential and microorganisms present. All of these factors interact to influence metal speciation, bioavailability and the overall toxicity of metals in the environment.

Persistence in environment

Because of their special chemical nature, metals are not as amenable as organics are. Unlike organics, metals are persistent in the environment and can not be degraded through biological, chemical or physical means to an innocuous byproduct. The chemical nature and, thus bioavailability of a metal can be changed through oxidation or reduction; however elemental nature

remains same because metals are neither thermally decomposable nor microbiologically degradable. Consequently metals are difficult to remove from environment.

Bio-toxicity of heavy metals

Living plants have the ability to accumulate heavy metals from soil and water (Miller, 1996; Boyajian and Carrecira, 1997). The metal uptake from soil by plants, through their roots, to both their above ground and underground parts depend on amount of metals present in the soil in exchangeable form. Moreover, the metal uptake from soil by plants is regulated by ability of plants to transfer the metals across the soil-root interface.

Plants distribute metals internally in many different ways. They may localize metals mostly in roots and stems or they may accumulate and store them in nontoxic forms for later distribution and use. A mechanism of tolerance or accumulation in some plants apparently involves binding of potentially toxic metals at cell walls of roots and leaves, away from sensitive sites within the cell or storing them in vacuolar compartment.

Some heavy metals at low doses are essential micronutrients for plants, but in higher doses they may cause metabolic disorders and growth inhibition for most of plant species (Fernandes and Henriques, 1991; Claire et al., 1991). Even though some plant species are endemic to metalliferous soils and can tolerate greater than normal levels of heavy metals or other toxic compounds (Banuelos *et al.*, 1997; Blaylock and Huang, 2000; Raskin and Ensley, 2000; Dahmani Muller *et al.*, 2000).

CHROMIUM

Chromium is a transition element located in the group VI-B of the periodic table with a ground state electronic configuration of Ar $3d^5 4s^1$. Chromium can occur in various valence (oxidation) states but Cr (III) and Cr (VI) are the only stable forms. Chromium is found in all phases of the environment including air, water and soil. Chromium content in natural soils ranges from 10-50 mg/kg of soil depending on parent material. In serpentine soil it can reach up to 125g/kg (Adriano, 1986).

Chromium and its compounds have diverse industrial uses. They are employed in leather processing and finishing (Nriagu, 1988), in the production of refractory steel, drilling muds, electroplating cleaning agents, catalytic manufacture and in production of chromic acid. Cr (VI) compounds are used in industry for metal plating, cooling tower water treatment, tanning and wood preservation. These anthropogenic activities have led to chromium contamination of environment.

Toxicity

Chromium is a toxic, nonessential element to plants. Hence plants are lacking a proper mechanism for its uptake. Carriers of other essential elements take part in its uptake from soil. The toxic effects of chromium are primarily dependent on the metal speciation determining its uptake, translocation and accumulation. Chromium is transported by way of an active mechanism involving carriers of elements like sulfate (Cervantes *et al.*, 2001).

Chromium is toxic even at low concentrations and is reported to cause severe oxidative damage to plant cells. It can effect growth, water balance, pigment content and initiate lipid peroxidation causing oxidative damage to plants (Bonet *et al.*, 1991; Poschenrieder *et al.*, 1993; Panda and Patra, 2000). A wide range of abiotic stresses including heavy metals produce, directly or indirectly toxic ROS (reactive oxygen species) like H_2O_2, $O_2^{\cdot}$, $OH^{\cdot}$ etc. Accumulation of chromium in different plant organs can reduce growth, induce chlorosis in young leaves, reduce pigment content, damage root cells and cause ultra structural modifications of the chloroplast and cell membrane (McGrath, 1995; Panda and Patra, 1997, 1998, 2000; Panda and Dash, 1999; Panda *et al.*, 2002, 2003; Panda, 2003;; Han *et al.*, 2004; Choudhury and Panda, 2005).

RATIONALE OF THE STUDY

It is evident from the review of literature that most of the work done on plant –heavy metal interaction was done on single species. Few studies have dealt with multi-species systems or communities as a whole. The results on single species studies can, however, be synthesized into a hypothesis to understand and explain the community responses to heavy metal contamination. Therefore, the primary objective of this study was to formulate, on the basis of current knowledge, a hypothesis to explain the response to communities to heavy metal contamination and to test experimentally at least some points of the hypothesis.

Chapter-2
Review of Literature

REVIEW OF LITERATURE

HEAVY METALS AND PLANT COMMUNITY STRUCTURE

Anthropogenic pollutants enter the environment in a variety of ways, which include mining, metal smelting, electroplating, gas exhaust, energy and fuel production, down wash from power lines, intensive agriculture, power transmission, sludge dumping and military operations (Kumar *et al.*, 1995; Nedelkoska and Doran, 2000). These anthropogenic pollutants include a variety of chemicals like heavy metals, phenolics, dust, oxides of sulfur, nitrogen and carbon, agricultural runoff, pesticides and organic explosives (Muller *et al.*, 1989; Van Asche and Clijsters, 1990). This contamination may result from mining operations, ore smelting, electroplating industry, use of heavy metal based dyes in dyeing and tanning industry, application of heavy metal containing pesticides, dumping of scrap metal and probably ship breakage industry.

As discussed in introduction, heavy metals are natural components of lithosphere. Soil or water, which contain heavy metals in excess of natural concentrations, are said to be heavy metal contaminated. Heavy metals are generally toxic above a threshold level and can be transferred to and concentrated in plant tissues from the soil media. Their toxicity to plants manifests itself in various ways and may become a health hazard to man and animals. The threshold level up to which animals and plants can tolerate heavy

metals varies with organisms and heavy metal ion species. Beyond the threshold level and even over a narrow range, heavy metals become toxic (Babich and Stotzky, 1980; Babich *et al.*, 1982). Moreover, these toxic metals adversely affect natural microbial populations leading to the disruption of vital ecological balance (Sterritt and Lester, 1980; Nriagu and Nieboer, 1988: Brynhildsen and Rosswall, 1997).

The excess concentrations of some heavy metals in soil such as Cd(II), Cr(VI), Cu(II), Ni(III) and Zn(II) have caused the disruption of natural aquatic and terrestrial ecosystems (Gardea Torresdey *et al.*, 1996; Meagher, 2000).

In a study of impact of heavy metals on plant diversity near a nickel-copper smelter in Kola Peninsula Russia, Koptsik *et al.* (2003) observed that the number, height and diameter of living trees and the crown density decreased towards the pollution source while the number of dead standing trees increased close to the smelter. Elevated concentrations of Ni, Cu, and S were found in the pine needles. The abundance of dwarf shrubs and lichens decreased with increase in metal deposition. Species richness declined from 13 to 5 per $100m^2$, the cover from 100 to 20%, and the phytomass decreased from 1.0 to 0.15 kg/m^2. Dwarf shrubs were found to be more stable while lichens like *Cladina stellaris* and *Cladonia rangiferina* were more sensitive to SO_2 or metals and did not occur in close vicinity of smelter. Variability in above ground phytomass and percent cover increased towards the smelter resulting in the formation of large barren patches.

Chernenkova and Kuperman (1999) studied the changes in spruce forest community along a heavy metal deposition gradient on Kola Peninsula. The number of under story plant species declined from 35 in the reference site (90.0 km from the smelter) to 6 in the site between 2.0 km and 5.0 km from the smelter. The decline was mainly due to reduction in the number of moss and lichen species. Most of the species were found between 20.0-90.0 km from the source. Only one lichen species (*Cladonia deformis*) occurred at 10.0km from the source. All the *Cladina* species were found beyond 20 km from the emission source. The percent cover and number of epiphytic lichen species decreased along the gradient as well. In contrast, there was an increase in the number of soil algae species, especially pollutant tolerant species of the genus *Chlamydomonas*, in more polluted sites. Only five angiosperm species (*Empetrum hermaphroditum, E. nigrum, Vaccinium myrtillus, V. vitis-idaea and Deschampsia fleruosa*) showed high biomass within 2.0-20.0 km of the source. It was argued that these tolerant species occupied the niches vacated by sensitive species.

Chiarucci *et al.* (1999) reported a significant increase in biomass production as a result of nutrient addition on a serpentine soil and concluded that the serpentine vegetation was mainly affected by nutritional stress rather than soil heavy metal content.

Gillet and Ponge (2003) analyzed the vegetation of three sites within a 20 year old poplar field, in the vicinity of a zinc smelter. Total zinc content of

topsoil varied from 4000 to 35,000 mg/kg according to the distance from the smelter outlet. Changes in humus forms (shift from mull to mor) and in degree of opening of the poplar stand (mainly caused by death or stunning of trees in the vicinity of the smelter) explained most of the observed variation in abundance and species composition. Some species living at the ground surface appeared at the most polluted sites where poplar declined and was replaced by a sward of metal tolerant herb species.

Heavy metals and lichen community

Lichens are known to be more sensitive to pollution than dwarf shrubs (Koptsik *et al.*, 2003). Some lichen species (*Cladina stellaris, Cladonia rangiferina*) were found to be more sensitive to SO_2 or heavy metals and did not occur near the nickel smelter (Koptsik *et al.*, 2003).

Niboer *et al.* (1972) emphasized the potential of lichen and epiphytes as the most useful indicators of heavy metal deposition around industrial plants. Kashulina *et al.* (1997) suggested that the disappearance of the ecologically important (protective) moss-lichen layer leads to frost damage to trees and dwarf shrubs, water and nutrient deficiency, soil erosion and may be viewed as the starting mechanism for severe phytocoenosis degradation.

Heavy metals and algal communities

Algal communities are major contributors to the energy transduction in aquatic food chains. Any disturbance in algal community can alter the structure of entire food chain and affect the functioning of whole ecosystem. Many workers

have focused their attention on the changes in algal communities caused by heavy metal contamination of water bodies.

In a study of diatom communities grown on medium containing Fe, Cu, Cr, Pb, Zn and crude oil (Coccetti & Lee, 1979), an alteration of community structure was observed. Growth of some species was depressed while that of others was enhanced. Greatest species diversity was consistently found in the community incubated in the presence of Cu. The trajectories of the communities enriched with oil, Pb, Cu and Cr were initially similar but latter diverged. The Fe enriched community, generally diverged from all others.

In a study of chemistry and vegetation of wet areas below the Elvins tailings pile in the Old Lead Belt of Missouri USA, high Zn levels (5.9-21 mg/l) were found in water (Whitton *et al.*, 1981). The two most widespread plant species were blue-green algae (*Plectonema gracillimum*) and a moss (*Dicranella* species). The latter existed predominantly in protonemal stage. In seepage studied in detail it was found that *Plectonema dicranella* protonema community predominated in the upper part of the seepage, which declined downstream.

In a study of the effects of Hg and Zn on algal community structure of Ganges water using CEPEX chambers, different algal groups showed different tolerance levels to these heavy metals (Rai *et al.*, 1990). Maximum inhibition of algal number was observed at 0.8 mug/ml of Hg followed by 8.0 mug/ml of Zn. Members of Chlorophyceae showed more tolerance than Cyanophyceae

and Bacillariophyceae. The filamentous forms were more tolerant to Hg and Zn than the unicellular forms.

Shehata *et al.* (1999) studied the toxic effects of mixture of metals, which existed simultaneously in aquatic ecosystem or natural phytoplankton assemblages (green algae, blue-green algae and diatoms). Substantial changes in phytoplankton community structure were detected and the most tolerant group was blue-green algae followed by green algae while diatoms were the most sensitive group. In all cases blue-green alga *Oscillatoria mugeotii* and green algae *Scenedesmuis gaudricauda* were most dominant species. Phytoplankton community was found to be most resilient. Metals were removed and accumulated by algae in following order: Zn > Cd > Ni > Cu > Cr.

Soldo and Behra, (2000) studied effects of long term Cu exposure on the community structure of fresh water periphyton. It was observed that 12-week Cu exposure (0.0, 0.05, 0, 1, 0.5, 1.0 and 5.0 mμM) affected the taxonomic composition of periphyton communities. Effects included shift in dominance from Cyanophyceae to Chlorophyceae. The relative abundance of *Oocystis nephrocytioides* increased from less than 1% in the control aquaria to 56% in the 5.0 mμM Cu treatments. Communities pre-exposed to 0.1 muM Cu showed significant increased tolerance to copper and co- tolerance to zinc, nickel and silver.

Paulsson *et al.* (2000) studied the changes in species composition and other parameters in periphyton communities exposed to various concentrations of zinc. Community structure was measured as species richness and Bray-Curtis similarly index. Marked changes in community species composition were found only at a higher concentration (9.7mμM).

Gold *et al.* (2002) studied the short-term effects of metal pollution by transferring periphyton diatoms communities from a reference site (unpolluted) to a site polluted by heavy metals (around 15 mμg Cd L^{-1} and 800 mμg Zn L^{-1}). A marked shift of community structure towards that of resident community of the polluted site was observed. A rapid change in taxonomic composition was observed due to decline in diatom density from 35000± 4000 to 15000±300 cells cm^{-3} after two weeks of transfer. Relative abundance of species characteristics of the reference sites, *Gomphonies minuta* and *Nitzschia dissipata,* rapidly decreased and species characteristics of the polluted site, *Gomphonema parvulum, Pinnularia* sp. or *Fragilaria crotonensis* progressively increased within transferred communities. It was concluded that shift in taxonomic periphyton diatom community structure could be an indicator of metal pollution under relatively low metal exposure.

Cunningham *et al.* (2005) studied the effects of anthropogenic contaminants and environmental variables on the composition of benthic diatom communities within a bay adjacent to an abandoned waste disposal site in Antarctica. Chemical data, particularly metal concentrations, explained 45.9

% variation in the diatom communities once the effect of grain size and spatial structure was excluded. Among the metals, tin (Sn) explained the greatest proportion of variation (28%) in the diatom communities. Tin was very highly correlated (R-2>0.95) with several other variables (copper, iron, lead and sum of metals), all of which explained similarly high proportions of total variation. Grain size data explained 23% of variation and pure spatial component explained only 1.8 % of the total variance. Thus, metal concentration accounted for greater portion of compositional variability in benthic diatom community.

Heavy metals and fungal community

Lebedeva and Kanivets (1991) studied the microfungal complexes in soil polluted with Cu, Zn and Pb. They considered the dominance of phytotoxic microfungi in the community of toxin forming fungi as indication of the deterioration of the microbiological state of the soil. *Aspergillus fumigatus*, *A. niger*, *A. terreus*, *Penicillium corylophilus*, *P. funiculosum*, *P. notatum*, *P. vermiculatum* and *Trichoderma konongii* were dominant toxin forming fungal species.

A study was conducted to monitor the changes in the state of micromycetes complexes under the impact of soil pollution caused by fumes of Norilsk metallurgical Works in forest-tundra of Taimyr peninsula, Russia (Kirtsideli *et al.*, 1995). A change in the number, biomass, structure of complex, species diversity and productivity of microorganisms (including phytotoxins, cellulose destruction etc.) was noticed.

Heavy metals and non fungal Microbial Community

Dean and Mills (1989) did not find a pollution related effect on bacterial number (viable and total), heterotrophic activity, and resistances to Pb, or Cu, or species diversity as determined by the either the Shannon's index or rarefaction in a study of bacterial communities in a heavy metal polluted river. The lack of correlation between heavy metal concentration and resistance in the sediment bacterial community was found to be due, at least in part, to the high pH of the river water and the resultant reduction in heavy metal toxicity.

Dean (1991) studied the response of attached bacteria to two concentrations of Zn (0.1 and 0.5mg Zn L^{-1}) at an ambient pH (8.4) and pH (5.5). He concluded that pH could modulate Zn toxicity to the attached bacterial communities. All measures of community abundance were highest at low Zn level and high pH. Conversely low pH and high Zn level caused significant lowering of biomass and total bacterial number.

Kozdroj (1995) studied the impact of acute dose (single dose of 5000.0 mμg Cd or Cu/g) or chromic doses (first dose followed by 4 weekly treatments of 1150, 1300, 1450 and 1100mμg/g of either metal) on community structure of heterotrophic bacteria. No differences between the number of the heterotrophic bacteria were noted in two treatments. Microbial community structure was dominated by K-strategist species. However bacterial community distribution curves suggested the evolution of different populations of K-strategist during experiments.

Sewage sludge applications during 1966 to 1989 increased concentrations of Cd, Cr, Cu, Pb and Zn up to 76% and reduced soil pH from 6.1 to 5.8. Dahlin *et al.* (1997) found significant differences in the bacterial community structure as determined by phospholipid fatty acid (PLFA) patterns between the high sludge treatment, low sludge treatment and control. A reduction of 15-80% in numbers of *Rhizobium leguminosarum* cv. *trifolii* was observed.

Techniques of %G+C profiling and community hybridization were applied to assess the changes in microbial community structure in a soil experimentally polluted with Cd, Cu, Ni, and Zn and incubated for 34 months. Data were compared with phospholipid fatty acid profiles (Griffiths *et al.*, 1997). The results showed that microbial communities were different between (a) uncontaminated and metal contaminated soils (b) soils contaminated with Cu or with other metals (c) soils contaminated with Pb or Ni.

Kelly and Tate (1998) compared the size, activity and structure of microbial communities from remediated and contaminated soils in the vicinity of a zinc smelter. Microbial community structure varied with increasing contamination. Soil remediation resulted in a decrease in soluble metals and increase in viable microbial population size. Remediated soils also showed metabolic profiles that were more similar to the least contaminated soils, suggesting recovery of the microbial populations. It was inferred that the microbial community might be a useful indicator of changes in soil quality.

Pennanen *et al.* (1998) studied the effect of separate and combined applications of acid and heavy metals on a forest soil microbial community structure. The relative amount of branched PLFAs common to gram-positive bacteria increased with decreasing humus pH while the treatment with metals alone did not cause clear changes in microbial community structure. The combined treatment of metal and pH caused changes similar to those caused by pH alone. Therefore the response of the bacterial community to heavy metal contamination may largely depend on soil pH.

Kelly *et al.* (1999) conducted a long-term study (420 days) of the effect of addition of zinc and acid on soil microbe community structure in soil microcosm. After 15 days of incubation the proportion of zinc resistant bacteria increased from 0.08% to 0.75%. As indicated by PLFA profile, Zn treatment caused a decrease in AM fungi and actinomycetes.

Soil particle size may also be an important factor in determining the effect of heavy metals on soil microbial community. Kandeler *et al.* (2000) reported that in a soil, exposed to various levels of Zn, Cu, Ni, V and Cd for 10 years, the microbial biomass within the clay fraction was predominantly due to soil bacteria while fungi were predominant in coarse sand fraction.

Chander *et al.* (2001) reported an increase in ergesterol IFE-biomass C ratio with increasing heavy metal (Zn, Pb and Cu) load in soils indicating a shift in microbial community structure from bacteria dominated to fungi dominated.

Various amendments can ameliorate the impact of heavy metals on soil microbial community. Karaca *et al.* (2002) treated soils with Cd alone, Cd + sewage-sludge and Cd + PO_4 fertilizer for 112 days. Addition of P and sewage-sludge decreased the availability of Cd in the same order. Addition of these amendments also restored the populations of fluorescent *Pseudomonads* and soil fungi.

A study of forest soil microbial communities in heavy metal contaminated and remediated soils around a zinc smelter (Kelly *et al.*, 2003), revealed that high heavy metal levels caused a decline in mycorrhizal fungi, gram positive bacteria, fungi and actinomycetes as indicated by PLFA procedure. Post remediation analysis with the same technique suggested a recovery of microbial populations.

Schipper and Lee (2004) studied the soil microbial communities in ultramafic soils on which populations of six different plant species grew. Microbial communities under each vegetation type were distinct. Elevated metal concentrations had no direct impact on microbial diversity. They concluded that the metal concentration affected the microbial community indirectly -- by controlling the vegetation type. However, while admitting the primary role of vegetation type, the secondary role of heavy metals as a selection force favoring metal resistant populations cannot be ignored. The lack of any observed impact on microbial diversity consequent to increase in heavy metals is itself suggestive of the resistant nature of microbial populations.

Abaye *et al.* (2005) compared the microbial biomass and community structure in three samples of sandy loam arable soil that had received the applications of farmyard manure (FYM) (1942-1967), metal contaminated sewage sludge (1942-1961) and mineral fertilizer (NPK from 1942-2004). Concentrations of Cu, Ni and Zn in sewage sludge treated soils were less than current European Union limits but the soil Cd concentration was more than twice the permitted limit. Gram-negative counts were found to be 62-68% greater than gram-positive in the metal contaminated soil. In FYM treated soil the gram positive count exceeded the gram negative count. The results suggested a substantial change in microbial community due to heavy metal contamination.

Ranella *et al.* (2005) studied the bacterial diversity in soils contaminated with Mn, Zn or Cd, Ni sludge. The structure of bacterial community as determined by denaturing gradient gel electrophoresis (DGGE) was different in the sludge-amended soils as compared with the control. The most important changes were observed in the soils amended with high level Ni, Cd sludge.

Hery *et al.* (2005) compared the effect of addition of carbon and nitrogen sources to Neocaledonian mine spoils characterized by high nickel content (20,000.0 ppm) and very low carbon (0.2%) and nitrogen (0.01%) and an agricultural soil. After succinate and glucose additions, actinobacteria were found to represent 75% and 96% respectively of the bacterial community in

mine spoils. However, same treatment in agricultural soil favored the increase of Firmicutes, mainly *Bacillus sp.*

HEAVY METAL AND ANIMAL COMMUNITY STRUCTURE

Animals are of indisputable importance to maintain the ecosystem balance. Soil and aquatic animals are likely to be adversely affected by drastic changes in the abiotic environment. Several workers have demonstrated the toxic effect of soil and water contamination on faunal composition (Bengtsson and Rundgren, 1988; Hagvar and Abrahamsen, 1990; Bruus Pedersen *et al.*, 1999; Scott-Fordsmand *et al.*, 1999; Christopher and Lisa, 2002 and Carolina *et al.*, 2004 etc). Heavy metal contamination may have an indirect influence on animal communities by impairing the litter decomposition and altering habit characteristics (Bengtsson *et al.*, 1988; Gillet and Ponge, 2002).

Plenet and Gibert (1994) demonstrated that Zn and Cu contaminated sites harbored low number of individuals and taxa. Responses of different taxonomic groups were not similar.

Macroinvertebrate benthic communities were compared on two sites, upstream and downstream of a mine in Ichi-Kawa River, Japan. Before the closure of mine in 1973, concentrations of arsenic, copper and zinc were distinctly higher downstream than at the upstream site. Chironomidae and a mayfly, *Epiorus latifolium* were found to be dominant at contaminated site. At reference site, stenopsychidae dominated. After mine closure family richness of

benthic communities clearly increased at contaminated site and Stenopsychidae became dominant in 1996 (Wantanabe *et al.*, 2000).

Clements *et al.* (1988) compared the insect community structure in an experimental stream contaminated with copper and zinc (12.0 mμg/l) and in a river contaminated with same metals. In both cases a reduced number of taxa, number of individuals and abundance of most dominant taxa was observed at contaminated sites. Uncontaminated sites were dominated by *Ephemeroptera* and *Tanytarsini chironomids* whereas contaminated sites showed a dominance of *Hydropsychidae* and *Orthocladiini.*

Stamou and Argyropoulou (1995) studied the oribatid communities structure along a traffic gradient. The soil was contaminated with Cu, Pb and Zn. Species richness was low in polluted areas.

Spurgeon and Hopkin (1999) studied the total abundance and biomass of earthworms in four seasons (spring, summer, autumn and winter) at 14 sites along two transects from a primary lead/zinc/cadmium smelting works in U.K. Total abundance and biomass of earthworms decreased with proximity to the smelter. The earthworms were altogether absent at two sites closest to the pollution source and catches were significantly lower than control at further five sites (greater than 3km). Species like *Aporrectodea calaginosa* and *Allolobophora chlorotica* which were dominant at relatively clean distant sites were absent from the most contaminated soils. Reduced species richness resulted in lower Shannon-Weiner index and higher Berger-Parker dominance.

HEAVY METALS AND PLANT GROWTH

Elevated heavy metal concentrations in the soil can also influence crop growth. At higher concentrations they interfere with metabolic processes and inhibit growth, sometimes act as stressors, leading to plant death (Baker *et al.*, 1976; Hoffmann, 1983; Baker, 1987 and Schaller and Diez, 1991). Consequently, quality standards were established that determine threshold value of maximum heavy metal concentrations allowed in the plant tissue. The transfer of heavy metals from soil to plants is dependent on three factors (Brummer *et al.*, 1986):

(1) The total amount of potentially available elements (**quality factor**). (2) The activity as well as the ionic ratios of elements in the soil solution (**intensity factor**), and (3) the rate of element transfer from solid to liquid phase and plant roots (**reaction kinetics**)

The absorption of metals from the soil by plants is influenced by a variety of factors including pH, temperature, soil solution concentrations, the cation exchange capacity of the soil, organic matter content of the soil, the type and concentration of metal and the species of plants (Antosiewicz, 1992; Salim *et al.*, 1993). The metals enter the soil in the form of dissolved ions and move with the inflow of water appoplastically through the root hairs and into the cortex and are then translocated to other parts of plants (Punz and Sieghardt, 1993).

The levels of metals found in plants are often correlated to the levels present in the environment (Vesk and Allaway, 1997). Salim *et al.* (1993)

showed that the concentrations of different heavy metals like lead, cadmium, copper increased in radish plants (*Raphanus sativus*) when treated with increasing concentrations of these metals. Imran *et al.*, (2007) also showed the similar results for Aluminum and Cadmium in soybean (*Glycine max*).

Germination

Application of hexavalent chromium to germinating green gram (*Vigna radiate* L. Wilczek var. K851) seeds (Panda and Khan, 2002), decreased the germination at higher concentrations. Increase in Cr concentrations uniformly increased protein content indicating inhibition of protein hydrolysis and a decrease in proline content was observed.

Root and shoot growth

Decline in shoot and root length is a well-documented response of plants to heavy metals (Breckel, 1991; Goldbold and Kettner, 1991; Rout *et al.*, 1997; Tang *et al.*, 2001). Different heavy metals affect various plant species differently (Prasad *et al.*, 2001).

Karataglis (1987) studied the toxic effects of different heavy metals on the root growth inhibition in greek wheat (*Triticum aestium* cv. *Vergina*). The order of metal toxicity revealed was: copper > chromium > nickel > zinc > lead > cadmium > aluminium > iron. The effect of magnesium and manganese concentrations showed little effect on root growth on seedlings, which displayed no symptoms of chlorosis. Grubinger *et al.* (1994) observed plant

growth inhibition in Swiss chard (*Beta vulgaris* subsp. *cicla*) plants grown on soil amended with 10% and 15% rates of tannery waste.

The cadmium uptake by rice plants (*Oryza sativa*) on soil treated with different cadmium compounds revealed that the cadmium content of unpolished rice grown in soil treated with 50 ppm cadmium varied with the cadmium compounds applied (Muramoto *et al.*, 1991) and decreased in following order: cadmium chloride semipentahydrate, cadmium bromide tetrahydrate, followed by cadmium acetate dehydrate, cadmium oxide, cadmium cyanide, cadmium hydroxide, cadmium sulphide and cadmium carbonate. Treatment with cadmium iodide resulted in growth failure because of root tissue damage due to combined toxicity of cadmium and iodide ions.

Greger *et al.* (1991) studied the effect of acute dose of cadmium (single dose at relatively higher concentrations) and chronic dose (daily increments of 0.15or 0.20 mμmol) on sugar beet seedlings (*Beta vulgaris L.* cv *Monohill*) grown on a nutrient solution for 14 days. Cadmium caused growth retardation and increased root/whole plant ratio. The effects of cadmium were related to Cd^{2+} in the proportion both to the root absorption area and to the nutrient concentration.

Germination, initial growth, root development and metal accumulation by seedlings was studied in *Juncus acutus* treated with various concentrations of lead nitrate, copper sulphate and cadmium chloride (Stefani *et al.*, 1991). Germination was unaffected by all tested metals. Initial growth was strongly

inhibited by relatively higher concentrations of lead nitrate as compared to $CuSO_4$ and $CdCl_2$. The root inhibition was more pronounced than the shoot and failed to develop at all tested concentrations of $CuSO_4$. Seedling metal accumulation varied from 55% to 98% of the metals present in the culture solution. However, the accumulation of Cd in seedlings was higher than that of lead and copper.

Godbold and Kettner (1991) studied the effects of aluminium and lead toxicity, either supplied singly or in combination, on root elongation in *Picea abies*. Exposure to 50, 100, or 800 mμM, aluminium inhibited root elongation within 1 day. Complete recovery of root elongation was observed in 50 and 200 mμM treatments after 5 or 8 days respectively. At higher aluminium supply (800 or 1200 mμM) root elongation was inhibited over the duration of experiment. Relatively low levels of lead (0.5-2.0 mμM) also inhibited root elongation and no recovery was observed. Inhibition of root elongation by lead was lessened by the presence of aluminium.

Gorlach and Gambus (1989) studied the effects of five heavy metals (cadmium, copper, nickel, lead, and zinc) on Italian Ryegrass (*Lolium multiflorum*). Only copper (120mg/kg of soil) and zinc (320 mg/kg of soil) caused some disturbances in the growth. Copper caused a decrease in yield, while copper and zinc caused a decrease in root mass. Combined application of all five metals considerably increased the toxicity of Cu and Zn.

Gabbrielli *et al.* (1990) studied the effects of nickel on two serpentine species *Silene italica* and *Alyssum bertolonii*. The former species showed root growth inhibition and depressed mitotic activity in root tips at 7.5 mM concentration. *A bertolonii* remained unaffected by the same treatment. In *S. italica* an adequate calcium concentration (25 mM) was able to reverse the effect of nickel on root growth and metabolism. In *A. bertolonii*, the same calcium concentration reduced root growth, confirming the adaptation of this species to low calcium concentration, typical of serpentines.

In a study of root growth, mitotic activity and polypeptide pattern in the roots of *Lupinus leuteus* in presence of increasing concentrations of lead (Przymusinski *et al.*, 1991) an inhibition of root growth and reduced mitotic activity were observed. The most interesting observation was that Pb^{2+} quickly and selectively promoted the synthesis of some polypeptides while the production of others was reduced.

Lee *et al.* (1991) determined the growth and concentrations of 18 elements in current year foliage of *Pinus radiata* in a plantation on ultramafic soil. Correlation coefficients and multiple regressions of element concentrations against tree height indicated that copper was one of the main elements influencing *P. radiata* growth.

De-Vos *et al.* (1991) studied the relationship between copper tolerance and effect of copper on the plasmalemma of root cells using plants from one copper sensitive and two copper tolerant populations of *Silene cucubalus*. It

was shown that the damage to the permeability barrier of root cells constitutes the primary effect of copper toxicity in sensitive plants and that copper tolerance is coupled to the ability of the plants to prevent such damage. This ability might depend on exclusion of copper by the root cell plasamalemma.

Iqbal *et al.* (2001) demonstrated a significant reduction in shoot length elongation and germination of *Caeselpinia pulcherrima* in response to chromium treatment.

Gupta *et al.* (2005) treated *Brassia juncea* cv RH 30 with increasing doses of chromium (VI) (0.5, 1.0, 2.0, 4.0, 5.0, 7.5 and 10.0 ppm). The plants treated with 7.5 and 10.0 ppm concentrations showed 100% mortality within a week. Cr concentrations from 0.5-5.0 ppm inhibited the plant height, number of fruits, number of seeds per siliqua and seed yield per plant. A deterioration of oil quality was also observed as the treatments increased the erucic acid contents while the oleic, linoleic and linolenic contents decreased.

Khan *et al.* (2003) studied the impact of different Cr (0.0, 0.5, 0.8, 1.0, 2.0 and 4.0 ppm) and Cu (0.0, 0.2, 0.5, 0.8 and 1.0 ppm) concentrations on growth of maize (*Zea mays* L. cv Barnali). At highest concentrations of Cu (1.0 ppm) and Cr (4.0 ppm), the dry weight of root and shoot decreased by 82% and 65% for Cu and 82% and 68% for Cr, respectively.

Samantaray and Deo (2004) studied the effect of different concentrations (0.0, 24.0, 48.0, 96.0, 192.0 and 384.0mμM) of hexavalent chromium ($K_2Cr_2O_7$) on root and shoot of mung bean (*Vigna radiate* cv. PDM-

54) grown in hydroponic culture. No concentration was found to adversely effect the shoot elongation but root did not develop at 192 mμM Cr.

Zhou-Xiqin and Ji-Qiahua (2005) recorded gradual decrease in seed germination rate, plant height, root length, fresh weight and dry seedling biomass with increasing $CrCl_3$ concentration in *Zea mays*.

Scoccianti *et al.* (2006) studied the toxicity of Cr(III) in celery (*Apium graveolens*) seedlings and showed that Cr(III) induces deleterious effects on the seedling development morphology. Varied concentrations (from 0.01- 10.0 mM) increasingly inhibited seedling germination and hypocotyls elongation or completely blocked hypocotyls elongation (10.0 mM), while the root apparatus was damaged at the lowest dose.

Wani *et al.* (2007) studied the impact of heavy metal toxicity on chickpea (*Cicer arietinum*). Cadmium at 23.0 mg/kg soil, when used alone or in combination with other metals, was found to be the most toxic and significantly reduced the plant growth, nodulation, chlorophyll content and root and shoot N contents. The flowering was also delayed following the metal application. The degree of toxicity on measured parameters decreased in the following order: cadmium, zinc, nickel, copper, chromium and lead.

Garcia *et al.* (2006) studied the impact of metal- ion contaminants (Cd, Cu, Pb and Zn) on sunflower (*Helianthus annuus* L). Decrease in height and mass by 35% and 40% respectively in comparison to control was observed.

In a study of combined effects of Cd $^{2+}$ (0.0, 10.0, 100.0, 500.0, mμmol/L), acetochlor (AC) (0.0, 1.6, 4.0, 8.0, mμmol/L) and bensulfuronmethyl(BSM) (0.0, 0.16, 0.40, 0.80mμmol/L) on rice (*Oryza sativa* L.) cultivar Jinyou 402 (Huang *et al.*, 2006), it was observed that combined application of Cd and AC significantly inhibited the growth of the roots and shoots. The root dry weight /shoot dry weight (RDW/SDW) ratio, total chlorophyll content and chlorophyll a/b ratio decreased by 41%, 50%, 56% respectively, in comparison to control. Plant dry weight / plant fresh weight (PDW/PFW) ratio increased by 284% and 84% respectively. The application of combined treatment of Cd and BSM showed the similar results. The results suggest that the combination with herbicides enhanced the toxicity of Cd to rice seedlings.

Mediouni *et al.* (2006) studied tomato seedlings, cultivated in nutrient solution supplemented with increasing concentrations of $CdCl_2$ or $CuSO_4$ from 0.0 to 50.0 mμM. After 7-days of treatment, it was observed that Cu and Cd, decreased tomato growth, notably at high Cu levels.

Jain and Srivastava (2006) studied the effects of various concentrations of Cd (0.0, 0.5, 10.0, 100.0, 200.0 ppm as cadmium chloride) on sugarcane (*Sachharum officinarum* Hybrids COLK 8102 and COJ 64). All cadmium concentrations resulted in decrease in most of the growth parameters studied i.e., leaf number, leaf area, plant height, leaf width, fresh and dry weight of different plant parts.

Shute and Macfie (2006) studied the effects of Cd and Zn on soybean [*Glycine max (L.) Merr.*] either separately or in combination. Highest dose of Cd (100.0 mg/kg of soil) reduced plant height and dry weight down to 40% and 34% of control, respectively and the highest dose of Zn (2000.0 mg/kg of soil) reduced plant height to 55% and plant dry weight to 70% of control. Combined applications of the two metals elicited approximately similar response.

Khan and Siddhu (2006) studied the phytotoxic effects of Cd on urdbean [*Vigna mungo (L.) Hepper*] 10(-2) M concentration showed significant decrease in germination relative index (G.R.I.), length and dry weight of root and shoot, nodule number and chlorophyll content while 10(-8)M concentration was slightly promotive over control.

Prestes and Caires (2005) studied the effects of molybdenum and cobalt on soybean seeds. Application of Mo resulted in decrease in Fe content in the leaves but did not affect soybean yield. Co application resulted in significant linear decrease in plant height, leaf concentration of Zn and grain yield.

Fuentes *et al.* (2007) studied the effects of three heavy metals (Cu, Ni and Zn) on four Mediterranean woody seedlings (*Pinus halepensis, Pisticia lentiscus, Juniperus oxycedrus* and *Rhamnus alaternus*). Application of varying concentrations of heavy metals (0.048, 1.0 and 4.0 mμM of Cu; 0.0, 25.0 and 50.0 mμM of Ni; and 0.073, 25.0 and 100.0 mμM of Zn) in a hydroponic sand silica culture for 12 weeks The intermediate concentrations enhanced biomass accumulation, whereas the highest concentrations resulted in

reduction in biomass. Decrease in shoot biomass resulted at internal concentrations of ranging from 25.0 to 128.0 mμg /g of Zn and 1.7 to 4.1 mμg /g of Cu. *Rhamnus alaternus* and *Juniperus oxycedrus* showed higher sensitivity to Cu and Zn than *Pinus halepensis* and *Pistacia lentiscus*.

Plant biomass

Mehla *et al.* (1989) studied the Cd and Zn interaction in sorghum. In screen house experiment using 0.0, 5.0, 10.0, 20.0, 40.0 and 80.0 ppm Cd and 0.0, 5.0, 10.0, 20.0, 40.0, 60.0 and 80.0 ppm Zn, the dry matter yield of sorghum decreased markedly and consistently due to Cd application, while Zn had favorable effect. The dry matter yield decreased linearly with Cd content and an increase of 1.0 ppm Cd concentration in shoot caused a reduction of 0.668 to 1.857 g/pot dry weight depending on Zn supply.

Jasiewicz (1989) conducted a water culture experiment to asses the impact of varying doses of copper (0-100 mg/dm^3) on maize, wheat, sunflower, hemp and rape. Rape and hemp were most sensitive as they showed yield loss beginning with the dose 0.2mgCu/dm^3. Maize and sunflower and wheat were moderately tolerant showing yield loss from dose of 0.5 mg Cu/dm^3 onwards. The species showing high tolerance (maize and sunflower) accumulated relatively higher amounts of Cu in their tops (29.1 mg/kg dry mass and 11.0 mg respectively). Cu accumulation in shoots of rape and hemp (8.8mg and 6.2mg respectively) were the lowest. Cu accumulation by roots also showed same trend but the magnitude and accumulation was much higher than the shoots.

Wang and Yan (1990) studied the effects of the application of copper containing sludge on wheat and rice in calcareous soil. Rice was more susceptible than wheat showing a 10% yield reduction at 100.0 ppm Cu treatment. The Cu content in grains of both wheat and rice did not exceed 20.0 ppm. It was suggested that Cu content must not exceed 800.0 ppm in sludge used to fertilize calcareous soil.

Dang *et al.* (1990) evaluated the yield of onion (*Allium cepa*) and fenugreek (*Trigonela foenum-graecum*) as affected by the applications of cadmium, nickel, lead and zinc at the rate of 0.0, 50.0, 100.0, 200.0 and 400.0 mg/kg of soil. Cd was found to be the most toxic as it drastically reduced the fresh and dry weights of both crops at 50.0 mg/kg soil. Onion was more tolerant to heavy metals as compared with fenugreek.

Chao and Wang (1990) studied the effect of various concentrations of Zn, Cu, Ni, Cr, Pb and Cd on growth and mycorrhizal infection of roots of maize (*Zea mays*). A reduction in length and dry biomass of infected roots was observed.

In a study of effects of the application of Mn, Cu and Ni on mulberry seedlings (Lou *et al.,* 1991) an 84% reduction in dry weight was observed in Ni treated seedlings. Combined application of higher concentrations of Cu and Ni had a synergistic effect causing more significant decrease of dry matter yield than single application of either metal. Combined application of Mn and Ni did not show the synergistic effect.

Iqbal *et al,* (1991) reported a significant reduction in seed germination of *Leucaena leucocephala, Samanea saman* and *Dalbergia sissoo* with increasing concentrations of Cd. Maximum germination inhibition was recorded in *Dalbergia sissoo.* Seedling length and biomass also showed same trend.

Application of excess aluminium to young trees of *Pinus nigra* var. *maritima* (Boxman *et al.,* 1991) resulted in a simultaneous reduction of root and shoot biomass, decline of fine root system, an increase in the coarse/fine root ratio and a decrease in the degree of mycorrhizal infection. Reduction in the uptake of divalent cations (Ca, Mg, Fe, Mn and Zn) was also recorded.

Plant yield

Gupta and Potalia (1990) studied the impact of Cd and Zn on the yield of wheat. Zn application enhanced grain and straw yields and Cd application reduced yields drastically.

Muramoto *et al,* (1990) compared the sensitivity of wheat and rice to cadmium in the form of cadmium oxide. Application of Cd at 30.0 ppm decreased wheat yield by 30.0% while same treatment caused only 8.0 % reduction in yield of rice plants.

In a study of effect of Ni enriched sewage water on the yield of corn (*Zea mays*) Narwal *et al.* (1991) found that lower levels of nickel enhanced the corn yield. Higher Ni levels reduced the yield sharply. Maximum loss occurred at 200.0 ppm of Ni.

Sharma *et al.* (2003) studied the effect of chromium in *Zea mays* L cv Ganga 5. They observed visible lesions of interveinal chlorosis, vein clearing in young leaves and papery appearance of leaves. Reduction in both chlorophyll *a* and chlorophyll *b* was observed. The activities of ribonuclease and phenylphosphatase were greater in Cr exposed plants but the activity of Fe-porphyrin enzyme catalase and amylase was low. Reduction in soluble protein content was observed. Decline in grain production and quality was noticed.

Equitability change in a community in response to HM contamination has so far received little attention and most of the work is related to soil microbes, fungi and aquatic organisms (Sterritt and Lester, 1980; Nriagu and Nieboer, 1988; Brynhildsen and Rosswall, 1997; Coccetti & Lee, 1979; Rai *et al.*, 1990; Shehata *et al.*, 1999; Soldo and Behra, 2000; Paulsson *et al.*, 2000; Gold *et al.*, 2002; Cunningham *et al.*, 2005; Lebedeva and Kanivets ,1999; Kirtsideli *et al.*, 1995; Kelly and Tate,1998; Pennanen *et al.*, 1998; Kelly *et al.*, 999 etc. and many others cited in review of literature.).

Chapter-3

Materials & Methods

MATERIALS AND METHODS

The study was carried out in the Botanic Garden of Department of Botany, Aligarh Muslim University, Aligarh during the months December to April 2006-2007. The experiments were conducted in 25 cm earthen pots filled with garden soil. Local flora seed bank was obtained from the top 2.0 cm soil obtained from wasteland near the Botanic Garden. The soil was thoroughly mixed to get maximum homogeneity of seed bank. Equal amount of seed bank so obtained was evenly spread in each pot. The pots were put in a net house and watered as per requirement.

PREPARATION OF VARIOUS DOSES OF CHROMIUM NITRATE

Different Molar concentrations of chromium nitrate were prepared as follows:

S.No.	Weight of chromium nitrate (g) in 1000.0ml. solution	Molarity
1.	400.15	1.0
2.	200.075	0.5
3.	80.03	0.2
4.	40.015	0.1

Experiment -1

ACUTE TREATMENT

The aim of this experiment was to investigate the impact of acute chromium nitrate contamination on the plant community. 100 ml Chromium nitrate solutions of different concentrations (0.2, 0.5 and 1.0 M) were applied on March 2, 2007, after the germination was complete.

Experiment-2

CHRONIC TREATMENT

The aim of this experiment was to investigate the impact of chronic chromium nitrate contamination on the plant community. Three concentrations of 100 ml chromium nitrate (0.1, 0.2 and 0.5 M) were applied on March 2, March 7 and March 12, 2007. Each treatment was replicated five times.

Sampling

The sampling was done after maturation of the plants and following parameters were recorded:

1. Total dry weight of each species
2. Mean shoot length.
3. Mean root length.
4. Mean shoot dry weight.
5. Mean root dry weight.
6. Mean fruit number plant^{-1}.
7. Mean seed number plant^{-1}.

Root and shoot length

Root and shoot length were measured in centimeters with a measuring tape.

Dry weight of shoots and roots

The shoots and roots were dried in a hot air oven at 80^0 C for 24 hours and then weighed on an electrical balance.

Mean fruit and seed number plant^{-1}

The sum of number of fruits/seeds borne by the sample was divided by the sample size.

STATISTICAL ANALYSIS

The data were subjected to statistical analysis. Mean and standard deviation were applied as measure of central tendency and variability respectively. Student t-test was applied to test the significance of difference of means.

Mean ($\overline{X}$)

The mean was dividing the sum of observations by total number of observations, thus

$$\overline{X} = \frac{x_1 + x_2 + \text{----------} x_n}{N}$$

$$\overline{X} = \frac{\sum_{i=1}^{n} xi}{N}$$

Where x_1, x_2, ...x_n = observations

N= Total number of observations.

Percent variation (P.V.)

Percent variation is calculated to show and compare relative variability of two or more sets of measurements. Percent variation of any parameter, a unit less number, measures the magnitude of variation present between the mean of the control and any given treatment, relative to the control. It was obtained as:

$$P.V. = \frac{\overline{X}_T - \overline{X}_C}{\overline{X}_C}(100)$$

$\overline{X}_C$ and $\overline{X}_T$ are arithmetic means of parameters at control and treatment respectively.

Standard deviation (S.D.)

Standard deviation is the positive square root of the average of sums of squares of deviations of all observations from their mean. It was computed follows.

$$S.D. = \sqrt{\frac{(x_1 - \overline{x})^2 + (x_2 - \overline{x})^2 + (x_3 - \overline{x})^2 + --- + (x_n - \overline{x})^2}{n}}$$

$$\text{or } S.D. = \sqrt{\frac{\sum(x - \overline{x})^2}{n}}$$

Where, S.D. = Standard deviation

$\sum(x - \overline{x})$ = Sum of the deviations of all individual observations from their mean

i.e. $(x_1 - \overline{x}) + (x_2 - \overline{x}) + (x_3 - \overline{x})$----------$(x_n - \overline{x})$.

$\overline{X}$ = Mean of all observations

n = number of observations

Test of significance

The significant difference between treated and control population means was obtained by using the Least Significant Difference (L.S.D.) method. It was computed as follows.

Step 1: Construction of data table for 4 treatments and 5 replicates:

The data were computed such that each treatment occupied a column and their replicates were arranged in rows.

Rows (replicates	**Column number (treatments)**				**Total of rows (replicates) (Σ)**	**Square of total of rows**
	T1	**T2**	**T3**	**T4**		
R_1	A_1	B_1	C_1	D_1	$A_1+...+D_1=x_1$	$(x_1)^2$
R_2	A_2	B_2	C_2	D_2	$A_2+...+D_2=x_2$	$(x_2)^2$
R_3	A_3	B_3	C_3	D_3	$A_3+...+D_3=x_3$	$(x_3)^2$
R_4	A_4	B_4	C_4	D_4	$A_4+...+D_4=x_4$	$(x_4)^2$
R_5	A_5	B_5	C_5	D_5	$A_5+...+D_5=x_5$	$(x_5)^2$
Total of column (Σ)	$A_1+...+A_5$ $=y_1$	$B_1+...+B_5$ $=y_2$	$C_1+...+C_5$ $=y_3$	$D_1+...+D_5$ $=y_4$	$(x_1)^2+...+(x_5)^2$ $=wr$	Grand total $y_1+...+y_4 =$ wx
Squares of total of columns	$(y_1)^2$	$(y_2)^2$	$(y_3)^2$	$(y_4)^2$	$(y_1)^2+...+(y_4)^2$ $= wy$	
Sum of squares total of column (Σ^2)	$(A_1)^2+...+$ $(A_5)^2=Z_1$	$(B_1)^2+...+$ $(B_5)^2=Z_2$	$(C_1)^2+...+$ $(C_5)^2=Z_3$	$(D_1)^2+...+$ $(D_5)^2=Z_4$	$Z_1+...Z_4=wz$	$x_1+...x_5$

Step 2: Correction factor (CF)

$$CF = \frac{(\text{Grand Total})^2}{t \times r}$$

$$\text{or } CF = \frac{(wx)^2}{t \times r}$$

where,

wx = Grand total

t = number of treatments

r = number of replicates

Step 3: Total sum of squares (SSQT)

This is the sum of squares of all the values in the table minus the correction factor

$$SSQT = [\ (A_1)^2 + (B_1)^2 + \ldots\ldots\ldots\ldots\ldots\ldots\ (D_1)^2\ ] - CF$$

Step 4: Sum of squares of treatments (SSQt)

$$SSQt = \frac{(y_1)^2 + (y_2)^2 + \ldots\ldots\ldots (y_4)^2}{r} - CF$$

$$\text{or } SSQt = \frac{wy}{r} - CF$$

Where, r number of replicates

Step 5: Sum of squares of replicates (SSQr)

$$SSQr = \frac{(x_1)^2 + (x_2)^2 + \ldots\ldots\ldots (x_5)^2}{t} - CF$$

$$\text{or } SSQr = \frac{wr}{t} - CF$$

Where, t = number of treatments

Step 6: Sum of squares of error (SSQE)

SSQE = SSQT – (SSQt + SSQr)

Step 7: Estimated variance of error (MSE)

$$MSE = \frac{SSQE}{(t-1)(r-1)}$$

Step 8: Least significant difference based on students t-test (L.S.D.)

$$\text{L.S.D. at 5\% level} = \sqrt{\frac{2MSE}{r}} \times t \text{ value at 5\% level}$$

$$\text{L.S.D. at 1\% level} = \sqrt{\frac{2MSE}{r}} \times t \text{ value at 1\% level}$$

If the difference between any two sample means exceeds the least significant difference (L.S.D.) value obtained at 5% or 1% level, the difference between the two means is said to be significant at 5% or 1% level, respectively.

Chapter-4

Results

RESULTS

TOTAL DRY WEIGHT

Acute treatment

In community subjected to acute heavy metal treatment all species showed a trend of decrease in total dry weight. At 0.2 M concentration minimum dry matter decrease was shown by *Cynodon dactylon* (3.0%), followed by *Chenodpodium album* (3.9%), *Melilotus indica* (6.9%), *Coronopus didymus* (7.2%), *Spergula fallax* (8.0%), *Medicago polymorpha* (9.4%), *Veronica agrestis* (10.3%) and *Cyperus rotundus* (15.7%).

Treatment with 0.5 M concentration caused a loss of 6.0% in *C. dactylon*, 7.8% in *M. polymorpha*, 8.6% in *C. album*, 12.5% in *M. indica*, 12.6% in *S. fallax*, 15.7% in *C. rotundus*, 16.7% in *V. agrestis* and 17.3% in *C. didymus*.

Treatment with 1.0 M concentration caused a loss of 16.1% in *C. didymus*, 17.4% in *M. polymorpha*, 17.9% in *M. indica*, 19.7% in *S. fallax*, 19.9% in *C. dactylon* and *V. agrestis* 22.2% in *C. album* and 24.1% in *C. rotundus* (Table-1).

Chronic treatment

In community treated with chronic treatment of chromium nitrate all species showed similar trend of dry matter loss. Treatment with 0.1 M concentration caused a loss of 2.3% in *S. fallax*, 2.4% in *C. dactylon*, 4.5% in V.

Table 1: Effect of acute treatment of varying concentrations of chromium nitrate on total dry weight (g).

Plant species	Total dry weight				L.S.D. at 5%	L.S.D. at 1%
	Control	0.2 M	0.5 M	1.0 M		
Melilotus indica	7.02±0.78	6.53±0.76*	6.13±0.83**	5.76±0.62**	0.41	0.57
Medicago polymorpha	5.52±0.82	5.0±0.56**	5.09±0.67*	4.48±0.09**	0.35	0.49
Coronopus didymus	2.49±0.46	2.31±0.16*	2.06±0.18**	2.09±0.21**	0.16	0.22
Veronica agrestis	1.56±0.14	1.40±0.07**	1.30±0.09**	1.25±0.05**	0.09	0.12
Cynodon dactylon	1.66±0.07	1.61±0.09	1.56±0.12**	1.33±0.11**	0.08	0.11
Spergula fallax	2.13±0.19	1.96±0.11*	1.84±0.04**	1.71±0.81**	0.17	0.24
Chenopodium album	7.67±0.01	7.37±0.15	7.01±0.12**	5.97±0.43**	0.38	0.53
Cyperus rotundus	1.08±0.99	0.91±0.08**	0.91±0.08**	0.82±0.04**	0.06	0.08

Mean ± SD
M- Molar concentration.
'L.S.D.' Least Significant Difference.
'*' Significant at 5% Level and '**' Significant at 1% Level.

agrestis, 4.8% in *C. didymus*, 5.4% in *M. polymorpha*, 6.8% in *M. indica* and 7.4% in *C. rotundus*. *C. album* showed a marginal increase of 0.6%.

Treatment with 0.2 M concentration caused a loss of 3.9% in *C. album*, 6.5% in *C. rotundus*, 7.0% in *S. fallax*, 9.4% in *M. indica*, 9.6% in *C. dactylon*, 10.4% in *C. didymus*, 13.2% in *M. polymorpha* and 14.7% in *V. agrestis*.

Treatment with 0.5 M concentration caused a loss of 10.3% in *S. fallax*, 12.6% in *C. dactylon*, 17.4% in *M. indica*, 17.6% in *C. album*, 18.1% in *C. didymus*, 19.2% in *V. agrestis*, 19.4% in *C. rotundus* and 21.9% in *M. polymorpha* (Table-1A).

Shoot length

The data corresponding to the shoot length of the plant species in response to the acute treatment of varying concentrations of chromium nitrate solution are shown are Table-2. A progressive decline in shoot length was observed with increase in concentration of chromium nitrate solution in all the plant species. At 0.2 M chromium nitrate maximum loss in shoot length was recorded in *Veronica agrestis* (-17.04%). In community treated with 0.5 M chromium nitrate solution, all plant species showed loss in shoot length. Maximum loss was recorded for *Veronica agrestis* (-32.08%). Treatment with 1.0 M chromium nitrate also caused decrease in shoot length in all species. Maximum decrease was recorded for *Veronica agrestis* (-39.81%). The reduction in shoot length was statistically significant in all species at 1.0 M concentration at 5% as well as 1% confidence levels, except *Coronopus*

Table 1A: Effect of chronic treatment of varying concentrations of chromium nitrate on total dry weight (g).

Plant species	Total dry weight				L.S.D. at 5%	L.S.D. at 1%
	Control	0.1 M	0.2 M	0.5 M		
Melilotus indica	7.02±0.78	6.54±0.63*	6.35±0.76**	5.79±0.48**	0.37	0.52
Medicago polymorpha	5.52±0.82	5.22±0.21	4.79±0.49**	4.31±0.18**	0.33	0.47
Coronopus didymus	2.49±0.46	2.37±0.15	2.23±0.09**	2.04±0.17**	0.14	0.20
Veronica agrestis	1.56±0.14	1.49±0.05	1.33±0.12**	1.26±0.09**	0.08	0.11
Cynodon dactylon	1.66±0.07	1.62±0.12	1.5±0.11**	1.45±0.08**	0.06	0.09
Spergula fallax	2.29±0.38	2.08±0.12	1.98±0.16*	1.91±0.08**	0.13	0.18
Chenopodium album	7.67±0.01	7.72±0.12	7.37±0.15	6.32±0.15**	0.33	0.46
Cyperus rotundus	1.08±0.99	1.0±0.02**	1.01±0.12**	0.87±0.05**	0.05	0.07

Mean ± SD
M- Molar concentration.
'L.S.D.' Least Significant Difference.
'*' Significant at 5% Level and '**' Significant at 1% Level.

Table 2: Effect of acute treatment of varying concentrations of chromium nitrate on shoot length (cm).

Plant species	Shoot length				L.S.D. at 5%	L.S.D. at 1%
	Control	0.2 M	0.5 M	1.0 M		
Melilotus indica	59.28±3.62	52.64±3.71*	53.88±2.19	45.94±3.28**	5.49	7.70
Medicago polymorpha	49.88±6.84	47.4±3.72	41.62±3.13**	36.28±2.19**	3.27	4.59
Coronopus didymus	30.90±3.08	29.08±3.45*	27.22±2.30**	25.06±2.43**	1.67	2.34
Veronica agrestis	21.60±3.79	17.92±1.63*	14.67±2.07**	13.00±2.69**	2.45	3.73
Cynodon dactylon	17.10±3.54	15.46±3.21*	13.52±2.92**	13.88±1.59**	1.48	2.08
Spergula fallax	28.60±6.45	24.14±3.82*	22.40±4.14**	18.4±2.32**	3.51	4.93
Chenopodium album	52.75±7.85	50.82±3.14	45.56±4.49**	36.28±2.19**	4.05	5.68
Cyperus rotundus	24.60±2.20	21.74±4.49*	18.23±5.37**	16.08±3.72**	2.63	3.69

Mean ± SD
M- Molar concentration.
'L.S.D.' Least Significant Difference.
'*' Significant at 5% Level and '**' Significant at 1% Level.

didymus in which shoot length reduction was significant at 5% level only and *Veronica agrestis* in which it was significant neither at 5% nor at 1% level. Fig.1 shows the % loss of shoot length of various species as caused by 1.0 M chromium nitrate.

The data corresponding to the shoot length of the plant species in response to the chronic treatment of varying concentrations of chromium nitrate solution is shown in Table-2A. A progressive decline in shoot length was observed with increase in concentration of chromium nitrate solution in all the plant species. At 0.1 M chromium nitrate maximum increase in shoot length was recorded in *Cynodon dactylon* (30.76%), while maximum loss in shoot length was recorded in *Coronopus didymus* (-10.94%). In community treated with 0.2 M chromium nitrate solution, all plant species showed loss in shoot length. Maximum loss was recorded for *Melilotus indica* (-24.93%). Treatment with 0.5 M chromium nitrate also caused decrease in shoot length in all species. Maximum decrease was recorded for *Veronica agrestis* (-33.93%). The reduction in shoot length was statistically significant in all species at 0.5 M concentration at 5% as well as 1% confidence levels, except *Chenopodium album* in which shoot length reduction was significant at 5% level only and *Cynodon dactylon* in which it was not significant at both 5% as well as 1% levels. Fig.1 shows the % decline of shoot length of various species as caused by 0.5 M chromium nitrate.

Table 2A: Effect of chronic treatment of varying concentrations of chromium nitrate on shoot length (cm).

Plant species	Shoot length				L.S.D. at 5%	L.S.D. at 1%
	Control	0.1 M	0.2 M	0.5 M		
Melilotus indica	59.28±3.62	58.28±5.78	44.50±3.26**	45.8±3.45**	3.94	5.53
Medicago polymorpha	49.88±6.84	46.52±4.04*	41.56±3.58**	37.07±2.14**	3.27	4.58
Coronopus didymus	30.90±3.08	27.52±2.92**	25.10±1.37**	22.58±2.58**	2.09	2.93
Veronica agrestis	21.60±3.79	20.75±3.65	19.46±1.66*	14.27±1.95**	1.99	2.79
Cynodon dactylon	17.10±3.54	22.36±3.81	16.72±2.82	15.1±2.19*	1.96	2.75
Spergula fallax	28.6±6.45	27.9±4.32	22.7±2.76**	20.3±1.41**	3.39	4.76
Chenopodium album	52.75±7.85	55.54±4.80	47.58±3.22**	42.95±3.01**	3.49	4.89
Cyperus rotundus	24.60±2.20	23.80±2.50	22.80±3.46	17.90±2.83**	1.97	2.76

Mean ± SD
M- Molar concentration.
'L.S.D.' Least Significant Difference.
'*' Significant at 5% Level and '**' Significant at 1% Level.

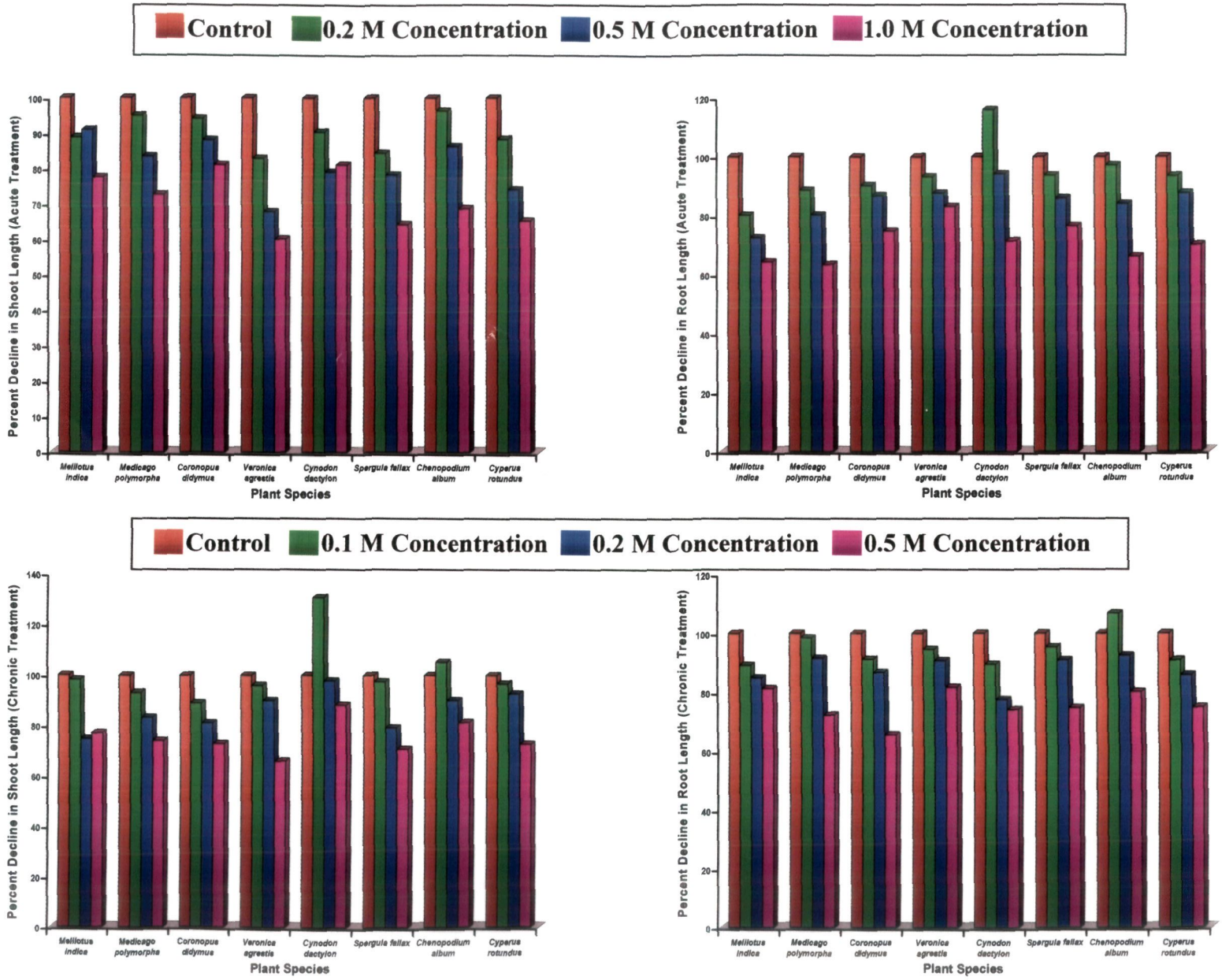

Fig. 1: Percent decline in shoot and root length of various plant species in response to acute and chronic chromium nitrate treatments.

Root length

The data corresponding to the root length of the plant species in response to acute treatment of varying concentrations of chromium nitrate solution is shown in Table-3. A progressive decline in root length was observed with increase in concentration of chromium nitrate solution in all the plant species. At 0.2 M chromium nitrate maximum increase in root length was recorded in *Veronica agrestis* (6.87%), while maximum loss in root length was recorded in *Melilotus indica* (-15.17%). In community treated with 0.5 M chromium nitrate solution, all plant species showed loss in root length. Maximum loss was recorded for *Melilotus indica* (-23.22%). Treatment with 1.0 M chromium nitrate also caused decrease in root length in all species. Maximum decrease was recorded for *Medicago polymorpha* (-39.81%). The reduction in root length was statistically significant in all species at 1.0 M concentration at 5% as well as 1% confidence levels, except *Cynodon dactylon* in which root length reduction was significant at 5% level only. Fig.1 shows the % decline of root length of various species as caused by 1.0 M chromium nitrate.

The data corresponding to the root length of the plant species in response to chronic treatment of varying concentrations of chromium nitrate solution is shown in Table-3A. A progressive decline in root length was observed with increase in concentration of chromium nitrate solution in all the plant species. At 0.1 M chromium nitrate maximum increase in root length was

Table 3: Effect of acute treatment of varying concentrations of chromium nitrate on root length (cm).

Plant species	Root length				L.S.D. at 5%	L.S.D. at 1%
	Control	0.2 M	0.5 M	1.0 M		
Melilotus indica	17.88±2.70	14.32±2.85**	12.96±1.59**	11.51±1.38**	1.57	2.20
Medicago polymorpha	18.42±2.57	16.34±2.37*	14.80±2.76**	11.70±1.38**	1.64	2.30
Coronopus didymus	15.32±2.18	13.84±0.39**	13.30±1.51**	11.46±1.65**	0.99	1.38
Veronica agrestis	9.02±1.08	8.40±1.27	7.90±0.50**	7.50±0.57**	0.62	0.87
Cynodon dactylon	10.52±1.30	12.20±2.04	9.92±1.25	7.52±0.91**	0.87	1.23
Spergula fallax	8.50±0.79	7.96±1.19	7.30±1.12**	6.50±1.03**	0.74	1.03
Chenopodium album	25.75±2.05	24.97±1.93	21.64±3.07**	17.04±1.81**	2.68	3.12
Cyperus rotundus	5.48±0.59	5.12±0.72	4.80±1.23*	3.84±0.58**	0.49	0.68

Mean ± SD
M- Molar concentration.
'L.S.D.' Least Significant Difference.
'*' Significant at 5% Level and '**' Significant at 1% Level.

Table 3A: Effect of chronic treatment of varying concentrations of chromium nitrate on root length (cm).

Plant species	Root length				L.S.D. at 5%	L.S.D. at 1%
	Control	0.1 M	0.2 M	0.5 M		
Melilotus indica	17.88±2.70	15.94±3.52*	15.18±2.36**	14.54±1.90**	1.38	1.94
Medicago polymorpha	18.42±2.57	18.14±1.95	16.88±1.98*	13.32±2.42**	1.43	2.0
Coronopus didymus	15.32±2.18	13.98±2.79	13.28±2.24**	10.05±0.72**	1.38	1.93
Veronica agrestis	9.02±1.08	8.52±0.71	8.18±0.72**	7.37±1.00**	0.52	0.73
Cynodon dactylon	10.52±1.30	9.42±1.24*	8.16±0.99**	7.80±0.97**	0.74	1.04
Spergula fallax	8.50±0.79	8.10±0.94	7.72±1.19	6.35±1.34**	0.83	1.16
Chenopodium album	25.75±2.05	27.46±2.29	23.64±2.41*	20.65±2.02**	1.91	2.68
Cyperus rotundus	5.48±0.59	4.98±0.52*	4.70±0.66**	4.10±0.59**	0.39	0.55

Mean ± SD
M- Molar concentration.
'L.S.D.' Least Significant Difference.
'*' Significant at 5% Level and '**' Significant at 1% Level.

recorded in *Chenopodium album* (10.46%), while maximum loss in root length was recorded in *Melilotus indica* (-10.85%). In community treated with 0.2 M chromium nitrate solution, all plant species showed loss in root length. Maximum loss was recorded for *Cynodon dactylon* (-22.43%). Treatment with 0.5 M chromium nitrate also caused decrease in root length in all species. Maximum decrease was recorded for *Coronopus didymus* (-34.40%). The reduction in root length was statistically significant in all species at 0.5 M concentration at 5% as well as 1% confidence levels, except *Melilotus indica* and *Chenopodium album* in which root length reduction was significant at 5% level only. Fig.1 shows the % decline of root length of various species as caused by 0.5 M chromium nitrate.

Shoot dry weight

The data corresponding to the shoot dry weight/plant of the plant species in response to the acute treatment of varying concentrations of chromium nitrate solution is shown in Table-4. A progressive decline in shoot dry weight/plant was observed with increase in concentration of chromium nitrate solution in all the plant species. At 0.2 M chromium nitrate maximum loss in shoot dry weight/plant was recorded in *Cyperus rotundus* (-15.55%). In community treated with 0.5 M chromium nitrate solution, all plant species showed loss in shoot dry weight/plant. Maximum loss was recorded for *Coronopus didymus* (-15.93%). Treatment with 1.0 M chromium nitrate also caused decrease in shoot dry weight/plant in all species. Maximum decrease

Table 4: Effect of acute treatment of varying concentrations of chromium nitrate on shoot dry weight (g).

Plant species	Shoot dry weight				L.S.D. at 5%	L.S.D. at 1%
	Control	0.2 M	0.5 M	1.0 M		
Melilotus indica	6.70±0.77	6.26±0.77*	5.90±0.83**	5.54±0.61**	0.40	0.56
Medicago polymorpha	5.22±0.80	4.72±0.57**	4.90±0.66	4.38±0.54**	0.33	0.46
Coronopus didymus	2.26±0.44	2.10±0.16*	1.90±0.16**	1.94±0.18**	0.14	0.20
Veronica agrestis	1.33±0.12	1.22±0.05**	1.16±0.06**	1.11±0.04**	0.07	0.09
Cynodon dactylon	1.42±0.08	1.38±0.06	1.35±0.11*	1.17±0.09**	0.06	0.09
Spergula fallax	1.96±0.18	1.82±0.08*	1.72±0.12**	1.61±0.07**	0.11	0.15
Chenopodium album	7.35±0.71	7.07±0.13	6.80±0.11**	5.76±0.39**	0.36	0.50
Cyperus rotundus	0.90±0.09	0.76±0.06**	0.76±0.07**	0.69±0.02**	0.05	0.07

Mean ± SD
M- Molar concentration.
'L.S.D.' Least Significant Difference.
'*' Significant at 5% Level and '**' Significant at 1% Level.

was recorded for *Cyperus rotundus* (-23.33%). The reduction in shoot dry weight/plant was statistically significant in all species at 1.0 M concentration at 5% as well as 1% confidence levels, except *Melilotus indica*, *Medicago polymorpha* and *Coronopus didymus* in which shoot dry weight/plant reduction was significant at 5% level only. Fig.2 shows the % decline of shoot dry weight/plant of various species as caused by 1.0 M chromium nitrate.

The data corresponding to the shoot dry weight/plant of the plant species in response to the chronic treatment of varying concentrations of chromium nitrate solution is shown in Table-4A. A progressive decline in shoot dry weight/plant was observed with increase in concentration of chromium nitrate solution in all the plant species. At 0.1 M chromium nitrate maximum increase in shoot dry weight/plant was recorded in *Chenopodium album* (+0.95%), while maximum loss in shoot dry weight/plant was recorded in *Cyperus rotundus* (-7.78%). In community treated with 0.2 M chromium nitrate solution, all plant species showed loss in shoot dry weight/plant. Maximum loss was recorded for *Veronica agrestis* (-14.29%). Treatment with 0.5 M chromium nitrate also caused decrease in shoot dry weight/plant in all species. Maximum decrease was recorded for *Medicago polymorpha* (-21.46%). The reduction in shoot dry weight/plant was statistically significant in all species at 0.5 M concentration at 5% as well as 1% confidence levels, except *Spergulan fallax* in which shoot dry weight/plant reduction was significant at 5% level only. Fig.2 shows the % decline of shoot dry weight/plant of various species as caused by 0.5 M chromium nitrate.

Table 4A: Effect of chronic treatment of varying concentrations of chromium nitrate on shoot dry weight (g).

Plant species	Shoot dry weight				L.S.D. at 5%	L.S.D. at 1%
	Control	0.2 M	0.5 M	1.0 M		
Melilotus indica	6.70±0.77	6.26±0.62*	6.10±0.75**	5.58±0.48**	0.36	0.50
Medicago polymorpha	5.22±0.80	4.94±0.20	4.56±0.50**	4.10±0.20**	0.32	0.45
Coronopus didymus	2.26±0.44	2.16±0.15	2.06±0.11**	1.86±0.07**	0.13	0.19
Veronica agrestis	1.33±0.12	1.30±0.07	1.14±0.11**	1.10±0.08**	0.08	0.11
Cynodon dactylon	1.42±0.08	1.38±0.11	1.28±0.11**	1.25±0.06**	0.05	0.08
Spergula fallax	1.96±0.18	1.92±0.13	1.85±0.08*	1.80±0.07**	0.08	0.11
Chenopodium album	7.35±0.07	7.42±0.16	7.12±0.16	6.12±0.15**	0.30	0.43
Cyperus rotundus	0.90±0.09	0.83±0.07*	0.85±0.11	0.71±0.10**	0.06	0.09

Mean ± SD
M- Molar concentration.
'L.S.D.' Least Significant Difference.
'*' Significant at 5% Level and '**' Significant at 1% Level.

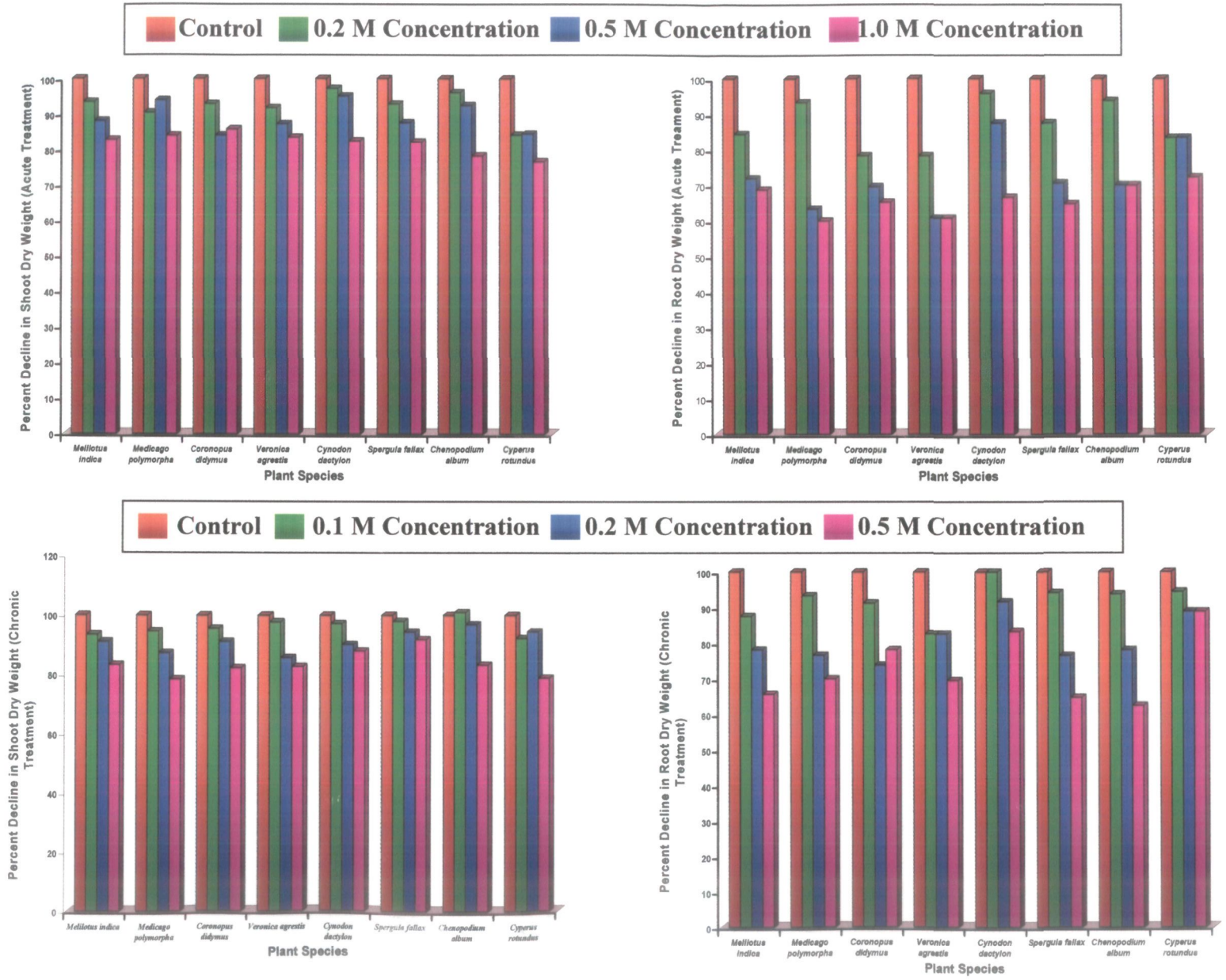

Fig. 2: Percent decline in shoot and root dry weight of various plant species in response to acute and chronic chromium nitrate treatments.

Root dry weight

The data corresponding to the root dry weight/plant of the plant species in response to the acute treatment of varying concentrations of chromium nitrate solution is shown in Table-5. A progressive decline in root dry weight/plant was observed with increase in concentration of chromium nitrate solution in all the plant species. At 0.2 M chromium nitrate maximum loss in root dry weight/plant was recorded in *Coronopus didymus* and *Veronica agrestis* (-21.74%). In community treated with 0.5 M chromium nitrate solution, all plant species showed loss in root dry weight/plant. Maximum loss was recorded for *Veronica agrestis* (-39.13%). Treatment with 1.0 M chromium nitrate also caused decrease in root dry weight/plant in all species. Maximum decrease was recorded for *Veronica agrestis* (-39.13%). The reduction in root dry weight/plant was statistically significant in all species at 1.0 M concentration at 5% as well as 1% confidence levels. Fig.2 shows the % decline of root dry weight/plant of various species as caused by 1.0 M chromium nitrate.

The data corresponding to the root dry weight/plant of the plant species in response to the chronic treatment of varying concentrations of chromium nitrate solution is shown in Table-5A. A progressive decline in root dry weight/plant was observed with increase in concentration of chromium nitrate solution in all the plant species. At 0.1 M chromium nitrate maximum loss in

Table 5: Effect of acute treatment of varying concentrations of chromium nitrate on root dry weight (g).

Plant species	Root dry weight				L.S.D. at 5%	L.S.D. at 1%
	Control	0.2 M	0.5 M	1.0 M		
Melilotus indica	0.32±0.03	0.27±0.02**	0.23±0.03**	0.22±0.03**	0.02	0.03
Medicago denticulate	0.30±0.04	0.28±0.03	0.19±0.02**	0.18±0.03**	0.03	0.04
Coronopus didymus	0.23±0.03	0.18±0.02*	0.16±0.02**	0.15±0.03**	0.04	0.05
Veronica agrestis	0.23±0.05	0.18±0.04**	0.14±0.04**	0.14±0.03**	0.03	0.04
Cynodon dactylon	0.24±0.04	0.23±0.04	0.21±0.04*	0.16±0.03**	0.02	0.03
Spergula fallax	0.17±0.03	0.14±0.03*	0.12±0.02**	0.11±0.03**	0.02	0.03
Chenopodium album	0.32±0.06	0.30±0.04	0.21±0.04**	0.21±0.05**	0.03	0.04
Cyperus rotundus	0.18±0.03	0.15±0.02*	0.15±0.02*	0.13±0.02**	0.02	0.03

Mean ± SD
M- Molar concentration.
'L.S.D.' Least Significant Difference.
'*' Significant at 5% Level and '**' Significant at 1% Level.

Table 5A: Effect of chronic treatment of varying concentrations of chromium nitrate on root dry weight (g).

Plant species	Root dry weight				L.S.D. at 5%	L.S.D. at 1%
	Control	0.1 M	0.2 M	0.5 M		
Melilotus indica	0.32±0.03	0.28±0.04*	0.25±0.03**	0.21±0.02**	0.03	0.04
Medicago polymorpha	0.30±0.04	0.28±0.05	0.23±0.04**	0.21±0.02**	0.03	0.04
Coronopus didymus	0.23±0.03	0.21±0.03	0.17±0.04**	0.18±0.04**	0.02	0.03
Veronica agrestis	0.23±0.05	0.19±0.03	0.19±0.04	0.16±0.04**	0.05	0.06
Cynodon dactylon	0.24±0.04	0.24±0.04	0.22±0.03	0.20±0.04**	0.02	0.03
Spergula fallax	0.17±0.03	0.16±0.04	0.13±0.04*	0.11±0.01**	0.03	0.04
Chenopodium album	0.32±0.06	0.30±0.04	0.25±0.05**	0.20±0.04**	0.03	0.05
Cyperus rotundus	0.18±0.03	0.17±0.02	0.16±0.03	0.16±0.03	0.02	0.03

M- Molar concentration.
'L.S.D.' Least Significant Difference.
'*' Significant at 5% Level and '**' Significant at 1% Level.

root dry weight/plant was recorded in *Veronica agrestis* (-17.39%). In community treated with 0.2 M chromium nitrate solution, all plant species showed loss in root dry weight/plant. Maximum loss was recorded for *Coronopus didymus* (-26.09%). Treatment with 0.5 M chromium nitrate also caused decrease in root dry weight/plant in all species. Maximum decrease was recorded for *Chenopodium album* (-37.50%). The reduction in root dry weight/plant was statistically significant in all species at 0.5 M concentration at 5% as well as 1% confidence levels, except *Cynodon dactylon* in which root dry weight/plant reduction was significant at 5% level only and *Cyperus rotundus* in which it was neither at 5% nor 1% level. Fig. 2 shows the % decline of root dry weight/plant of various species as caused by 0.5 M chromium nitrate.

Fruit number

The data corresponding to the fruit number/plant of the plant species in response to the acute treatment of varying concentrations of chromium nitrate solution is shown in Table-6. A progressive decline in fruit number/plant was observed with increase in concentration of chromium nitrate solution in all the plant species. At 0.2 M chromium nitrate maximum loss in fruit number/plant was recorded in *Coronopus didymus* (-21.33%). In community treated with 0.5 M chromium nitrate solution, all plant species showed loss in fruit number/plant. Maximum loss was recorded for *Melilotus indica* (-41.22%). Treatment with 1.0 M chromium nitrate also caused decrease in fruit

Table 6: Effect of acute treatment of varying concentrations of chromium nitrate on fruit number per plant.

Plant species	Fruit number				L.S.D. at 5%	L.S.D. at 1%
	Control	0.2 M	0.5 M	1.0 M		
Melilotus indica	672.00±72.94	585.00±43.59*	395.00±45.96**	307.00±56.82**	80.65	113.07
Medicago polymorpha	82.40±14.31	77.00±11.55	58.40±9.07**	42.33±3.79**	9.31	13.05
Coronopus didymus	422.00±32.90	332.00±19.20**	275.00±15.84**	211.00±17.13**	36.77	51.56
Veronica agrestis	108.00±13.73	98.60±12.14	76.00±6.48**	53.00±6.78**	11.60	16.26
Spergula fallax	54.60±12.58	47.40±7.50	35.00±2.03**	28.00±3.98**	8.02	11.25
Chenopodium album	202.00±21.09	179.00±8.22**	165.00±13.69**	141.00±8.22**	12.68	17.78

Mean ± SD
M- Molar concentration.
'L.S.D.' Least Significant Difference.
'*' Significant at 5% Level and '**' Significant at 1% Level.

number/plant in all species. Maximum decrease was recorded for *Melilotus indica* (-54.31%). The reduction in fruit number/plant was statistically significant in all species at 1.0 M concentration at 5% as well as 1% confidence levels. Fig.3 shows the % decline of fruit number of various species as caused by 1.0 M chromium nitrate.

The data corresponding to the fruit number/plant of the plant species in response to the chronic treatment of varying concentrations of chromium nitrate solution is shown in Table-6A. A progressive decline in fruit number/plant was observed with increase in concentration of chromium nitrate solution in all the plant species. At 0.1 M chromium nitrate maximum loss in fruit number/plant was recorded in *Melilotus indica* (-19.94%). In community treated with 0.2 M chromium nitrate solution, all plant species showed loss in fruit number/plant. Maximum loss was recorded for *Spergulan fallax* (-34.15%). Treatment with 0.5 M chromium nitrate also caused decrease in fruit number/plant in all species. Maximum decrease was recorded for *Coronopus didymus* (-46.68%). The reduction in fruit number/plant was statistically significant in all species at 0.5 M concentration at 5% as well as at 1% confidence levels. Fig.4 shows the % decline of fruit number of various species as caused by 0.5 M chromium nitrate.

Seed number

The data corresponding to the seed number/plant of the plant species in response to the acute treatment of varying concentrations of chromium nitrate

Table 6A: Effect of chronic treatment of varying concentrations of chromium nitrate on fruit number per plant.

Plant species	Fruit number				L.S.D. at 5%	L.S.D. at 1%
	Control	0.2 M	0.5 M	1.0 M		
Melilotus indica	672.00±72.94	538.00±69.58**	486.00±65.52**	402.00±32.13**	60.88	85.36
Medicago polymorpha	82.40±14.31	79.20±12.89	60.80±8.58**	49.00±6.67**	8.43	11.82
Coronopus didymus	422.00±32.90	364.00±28.90**	343.00±16.52**	225.00±11.34**	34.10	47.81
Veronica agrestis	108.00±13.73	100.00±10.46	79.80±11.82**	59.00±12.16**	10.94	15.33
Spergula fallax	54.60±12.58	49.00±7.07	36.00±6.56**	33.00±7.07**	8.37	11.73
Chenopodium album	202.00±21.09	186.00±7.42*	167.00±11.51**	148.00±10.95**	11.8	16.54

Mean ± SD
M- Molar concentration.
'L.S.D.' Least Significant Difference.
'*' Significant at 5% Level and '**' Significant at 1% Level.

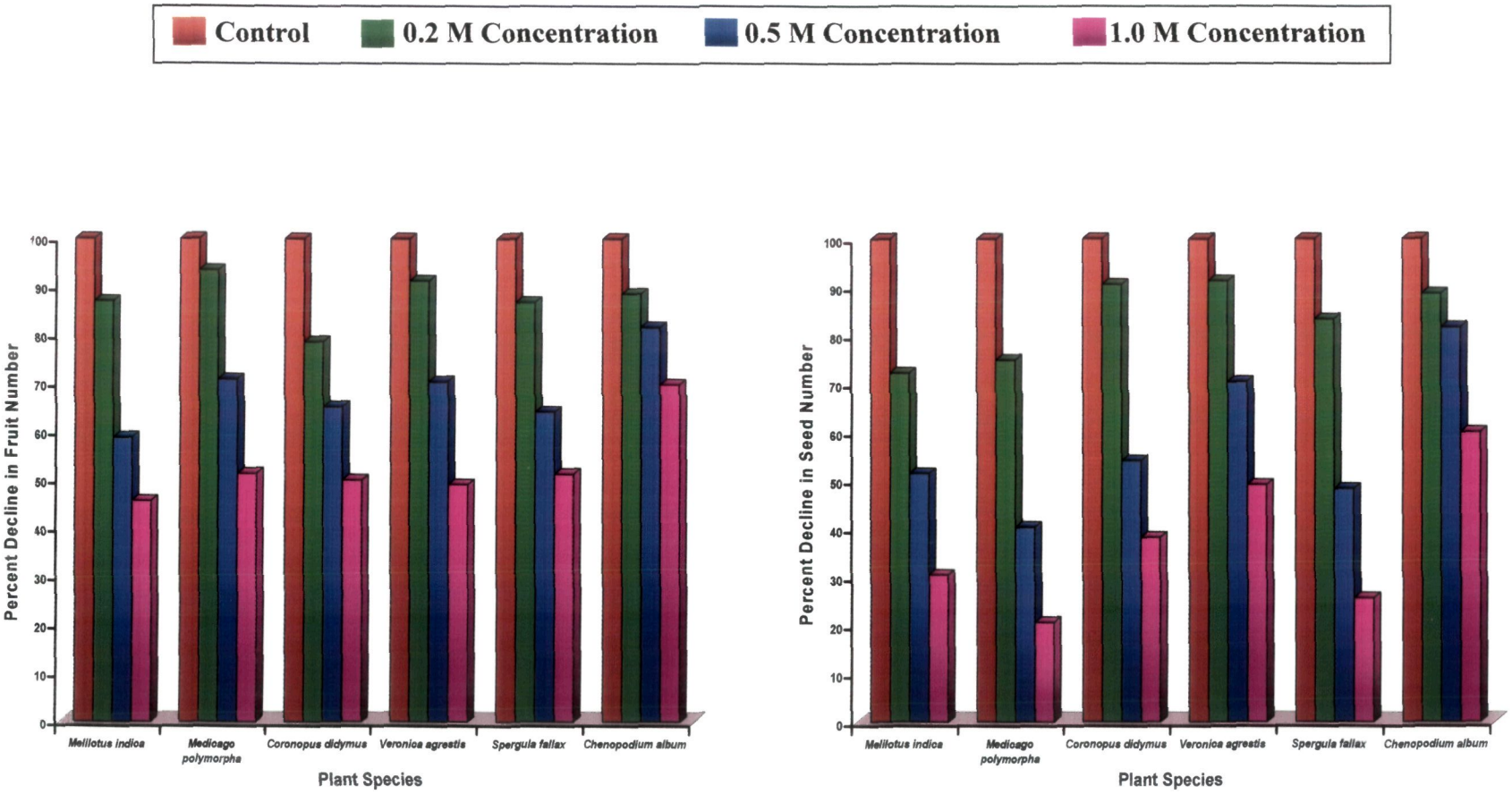

Fig. 3: Percent decline in mean fruit and seed number plant^{-1} in response to acute chromium nitrate treatments.

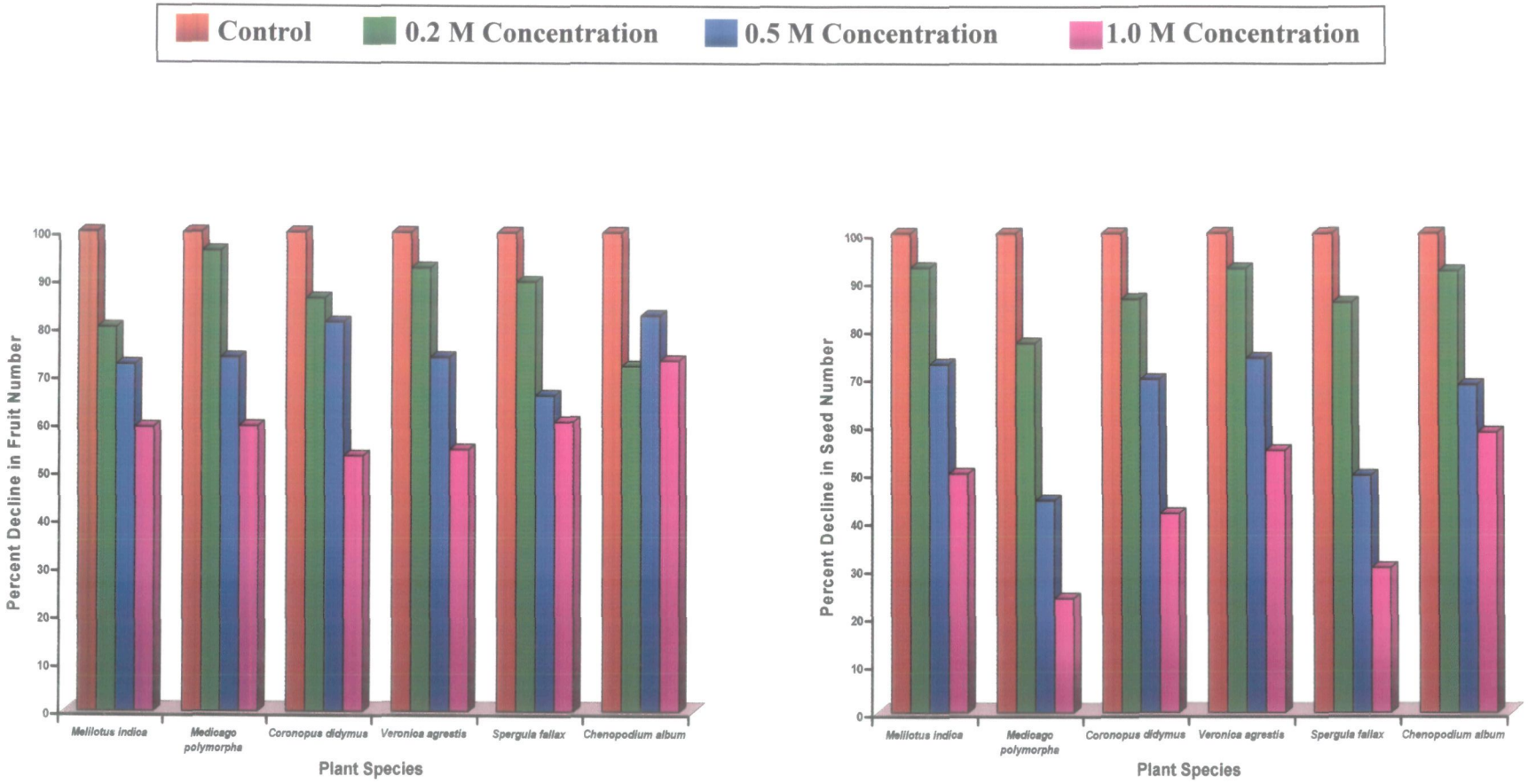

Fig. 4: Percent decline in mean fruit and seed number plant^{-1} in response to chronic chromium nitrate treatments.

solution is shown in Table-7. A progressive decline in seed number/plant was observed with increase in concentration of chromium nitrate solution in all the plant species. At 0.2 M chromium nitrate maximum loss in seed number/plant was recorded in *Melilotus indica* (-27.84%). In community treated with 0.5 M chromium nitrate solution, all plant species showed loss in seed number/plant. Maximum loss was recorded for *Medicago polymorpha* (-59.73%). Treatment with 1.0 M chromium nitrate also caused decrease in seed number/plant in all species. Maximum decrease was recorded for *Medicago polymorpha* (-79.41%). The reduction in seed number/plant was statistically significant in all species at 1.0 M concentration at 5% as well as 1% confidence levels. Fig.3 shows the % decline of seed number/plant of various species as caused by 1.0 M chromium nitrate.

The data corresponding to the seed number/plant of the plant species in response to the chronic treatment of varying concentrations of chromium nitrate solution is shown in Table-7A. A progressive decline in seed number/plant was observed with increase in concentration of chromium nitrate solution in all the plant species. At 0.1 M chromium nitrate maximum loss in seed number/plant was recorded in *Medicago polymorpha* (-22.96%). In community treated with 0.2 M chromium nitrate solution, all plant species showed loss in seed number/plant. Maximum loss was recorded for *Medicago polymorpha* (-55.74%). Treatment with 0.5 M chromium nitrate also caused decrease in seed number/plant in all species. Maximum decrease was recorded for *Medicago polymorpha* (-76.17%). The reduction in seed number/plant was

Table 7: Effect of acute treatment of varying concentrations of chromium nitrate on seed number per plant.

Plant species	Seed number				L.S.D. at 5%	L.S.D. at 1%
	Control	0.2 M	0.5 M	1.0 M		
Melilotus indica	1008.0±109.41	727.40±65.24**	518.0±57.44**	307.00±50.82**	133.76	187.53
Medicago polymorpha	205.60±35.77	154.00±23.11**	82.80±13.46**	42.33±3.79**	33.81	47.40
Coronopus didymus	422.00±32.90	382.00±19.20	228.00±17.20**	161.00±13.90**	42.13	58.98
Veronica agrestis	108.00±13.73	98.60±12.14	76.00±6.48**	53.00±6.78**	11.60	16.26
Spergula fallax	218.60±50.33	182.20±22.51	105.00±18.30**	56.00±7.52**	41.14	57.68
Chenopodium album	202.00±21.09	179.00±8.22*	165.00±13.69**	121.00±8.22**	16.01	22.45

Mean ± SD
M- Molar concentration.
'L.S.D.' Least Significant Difference.
'*' Significant at 5% Level and '**' Significant at 1% Level.

Table 7A: Effect of chronic treatment of varying concentrations of chromium nitrate on seed number per plant.

Plant species	Seed number				L.S.D. at 5%	L.S.D. at 1%
	Control	0.1 M	0.2 M	0.5 M		
Melilotus indica	1008.00±109.41	935.80±104.54**	732.00±81.09**	502.00±73.40**	117.37	164.55
Medicago polymorpha	205.60±35.77	158.40±25.78**	91.00±13.02**	49.00±6.67**	31.88	44.70
Coronopus didymus	422.00±32.90	364.00±28.90*	293.00±13.40**	175.00±15.20**	54.32	73.33
Veronica agrestis	108.00±13.73	100.00±10.46	79.80±11.82**	59.00±12.16**	10.94	15.33
Spergula fallax	218.60±50.33	187.00±21.21	108.00±19.67**	66.00±14.14**	47.50	66.59
Chenopodium album	202.00±21.09	186.00±9.42	138.00±10.95**	118.00±10.95**	17.51	24.51

Mean ± SD
M- Molar concentration.
'L.S.D.' Least Significant Difference.
'*' Significant at 5% Level and '**' Significant at 1% Level.

statistically significant in all species at 0.5 M concentration at 5% as well as at 1% confidence levels. Fig.4 shows the % decline of seed number/plant of various species as caused by 0.5 M chromium nitrate.

Chapter-5
Discussion

DISCUSSION

Physical environment of an ecosystem determines the type and composition of biological community. Therefore, any natural or anthropogenic modification of physical environment is bound to alter the community structure. Most of the studies addressing the question of changes in community structure, as evident from the review of literature, are based on soil microorganisms or algal and fungal communities. Studies on terrestrial plant communities are not only scanty, but most of them were conducted along long gradients of HM contamination. Present study attempts to record the changes in an artificial community in response to HM contamination in a uniform environment.

PLANT RESPONSE TYPES

Physiological responses of plants to heavy metal exposure are complex and bewildering. Same species responds to different heavy metals in different manners. Even various concentrations of a given metal can elicit different responses from same plant species. A combination of two or more metals may affect a plant in a manner that is entirely different from their individual impacts. Therefore, three response types, being proposed here, are purely ecological without any physiological implication. Following are three categories:

1. **VULNERABLE**: Those species, which fail to germinate or perish before reproducing.

2. **SENSITIVE**: Those species, which reproduce but their abundance, in terms of number of individuals or biomass is altered as also seed output.

3. **TOLERANT**: Those species, which show normal growth and reproduction.

HEAVY METAL CONTAMINATION AS A SELECTION FORCE

The categorization of plant species, as proposed above, implies that all species in a community will, most likely, not respond to heavy metal contamination in an identical manner. Vulnerable species being unfit to adapt to modified environment will be eliminated and result in decline of species richness of the community. In other cases an unfit subpopulation of a species may be eliminated leading to loss of genetic diversity. Sensitive species, though, are most likely to survive and stay in the community; however, their reduced biomass production will affect the equitability component of ecological diversity and productivity of the community as a whole. Clearly heavy metal contamination acts as a strong selection force favoring the species having physiological mechanisms to survive.

EFFECTS ON COMMUNITY PRODUCTIVITY

Integrity of a community can only be maintained if the ecosystem is functioning normally. Energy fixation and flow, nutrient cycling and regulation of organism by the environment and regulation of environment by the organisms are three major ecosystem functions. First two functions may be decidedly affected by heavy metal contamination.

Photosynthesis is the key process of energy transduction and a decline in its rate shall decline the amount of energy available in the community. Physiological studies have proved beyond any doubt that nearly all steps of photosynthetic process are adversely affected by heavy metals. Decline in chlorophyll content is a common response to HM contamination. This decline may result from inhibition of the biosynthesis of photosynthetic pigments (Mysliwa-Kurdziel *et al.*, 1999) or substitution of the central Mg in chlorophyll molecules by heavy metals (Hg, Cd, Cu, Ni, Zn, and Pb etc.). The HM substituted chlorophyll molecules fail to harvest the photons resulting in breakdown of photosynthesis (Küpper *et al.*, 1996, 1998). This study was, although, conducted on submerged aquatic plants but there is no reason why, at least, some terrestrial plants should not respond in the same manner. Enzymes involved in chlorophyll biosynthesis are also known to be inhibited by HMs, for example ALA synthetase by Cd (Stobart *et al.*, 1985 and Padmaja *et al.*, 1990), Pb (Burzy'nski, 1985); ALA dehydratase by Pb and Hg (Burzy'nski, 1985 and Prasad and Prasad, 1987 a & b), Cr (Vajpayee *et al.* 2000); porphobilinogenase by Mn, Fe, Co and Ni (Csatorday *et al.*, 1984 and Shalygo *et al.*, 1999); Uroporphyrinogen III decarboxylase by Cs (Shalygo *et al.*, 1997 and 1998), Co (Csatorday *et al.*, 1984) NADPH: protochlorophyllide oxidoreductase by Cd, Fe and Cr (Stobart *et al.*, 1985, Böddi *et al.*, 1995 and Berska *et al.*, 2001). Several studies have documented ultrastructural changes in chloroplasts, especially thylakoid membranes (Baszynski *et al.*, 1980;

Ghoshrony and Nadakavukaren 1990; Ouzounidou *et al.*, 1992; and Maksymiec *et al.*, 1994, 1995 etc.).

Besides these effects on constitution of photosynthetic machinery, key processes of photosynthesis e.g. harvesting of solar energy and dark reaction are also affected by HMs. Krupa (1988), Ahmed and Tajmir-Riahi (1993), Krupa and Baszynski (1995) reported the effect of heavy metals on light harvesting chlorophyll *a/b* protein complex. Miles *et al.*, (1972), Radmer and Kok (1974), Wong and Govindjee (1976) and Chugh and Sawhney (1999) reported the effect of Cu, Pb, and Zn on Photosystem I. Photosystem II is effected by Cd, Co, Cu, Hg, Ni, Pb and Zn (Miles *et al.* 1972; Bazzaz and Govindjee, 1974; Tripathy and Mohanthy, 1980; Hsu and Lee, 1988; Yruela *et al.*, 1991, 1993, 2000; and Vernay *et al.*, 2007 etc.). Heavy metals also affect Oxygen evolving complex, Plastoquinone pool, cytochrome *b6/f*, plastocyanin, ferredoxin, ferredoxin $NADP^+$ reductase and chloroplast coupling factor. All three key steps of Calvin cycle: carboxylation, reduction and regeneration are affected by HMs. Carboxylation appears to be most sensitive as several enzymes of this stage, for example Ribulose –1,5-biphosphate carboxylase/oxygenase (Kitao *et al.*, 1997; Siedlecka *et al.*, 1997, 1999; Subrahmanyam and Rathore, 2000 and Monnet *et al.*, 2001), PEP-carboxylase and 3-phosphoglyceric acid kinase (Sheoran *et al.*, 1990, Van Assche abd Clijsters, 1987, 1990 etc.), NADP dependent glyceraldhyde 3-phosphate dehydrogenase (Van Assche and Clijsters, 1987, 1990; Sheoran *et al.*, 1990) Fructose 1,6 biphosphatase (Sheoran *et al.*, 1990 and Malik *et al.*, 1992);

Aldolase (Sheoran *et al.*, 1990); Fructose 6-phosphate, 2-kinase (Malik *et al.*, 1992); Adenosine-diphosphate glucose pyrophosphorylase (Malik *et al.*, 1992) are affected by heavy metals.

Effect of HMs on respiration is not so predictable. The rate of respiration can increase as well as decrease in response to HM contamination. Lösch (1999) has listed a large number of references on HM induced changes in respiratory gas exchange. The respiratory gas exchange may be as low as 12% to as high as 180% of control. Apparently it may appear that slowing down of respiratory activity will result in increased net primary productivity if photosynthesis operates normally. It is, however, very unlikely that photosynthetic rate will remain unaltered if a plant is subjected to heavy metal stress. Moreover, declined respiratory rate will also decrease the quantum of energy available for over all metabolism.

SEED OUTPUT

Seed output ensures that sufficient seed bank will be available for next progeny. Decreased seed output means decline in soil seed bank. Species with a long seed viability period may recover if HM perturbation is for a short period, but species with short seed viability period will suffer a setback. Many studies cited above have also documented reduced seed output in response to HM contaminations. Alterations in seed output caused by HM contamination will also alter the relative proportion of various species in next generation. Moreover, decreased germination rate of a species with declined seed output will further shrink its population size.

IMPLICATIONS FOR COMMUNITY DIVERSITY

There are two components of community diversity (a) Species richness and (b) Equitability. The former refers to actual number of taxa in a community and the latter refers to the distribution of abundance values among the species either in terms of number or biomass of individuals of different species. In a stable community equitability remains more or less constant over generaions, but environmental perturbations are known to modify this component. HMs have been shown to affect both components of community. Several studies have demonstrated decline in number of taxa in HM contaminated areas in aquatic as well as terrestrial environments (Coccetti & Lee, 1979; Whitton *et al.*, 1981; Rai *et al.*, 1990; Chernenkova and Kuperman, 1999; Soldo and Behra, 2000; Paulsson *et al.*, 2000; Gold *et al.*, 2002; Gillet and Ponge, 2003; Koptsik *et al.* 2003; Cunningham *et al.*, 2005 etc.). Other studies have documented the changes in equitability component (Coccetti & Lee, 1979; Kirtsideli *et al.*, 1995; Chernenkova and Kuperman, 1999; Shehata *et al.*, 1999; Soldo and Behra, 2000; Paulsson *et al.*, 2000; Gold *et al.*, 2002; Koptsik *et al.* 2003; Gillet and Ponge, 2003; Cunningham *et al.*, 2005 etc.).

Fig.5 hypothesizes how heavy metal contamination can bring about changes in community structure. Salient points of the hypothesis may be summarized as follows.

1. Tolerant species or subpopulations are likely to remain unaffected without any adverse affect on germination, photosynthesis and respiration and consequently normal growth and reproduction.

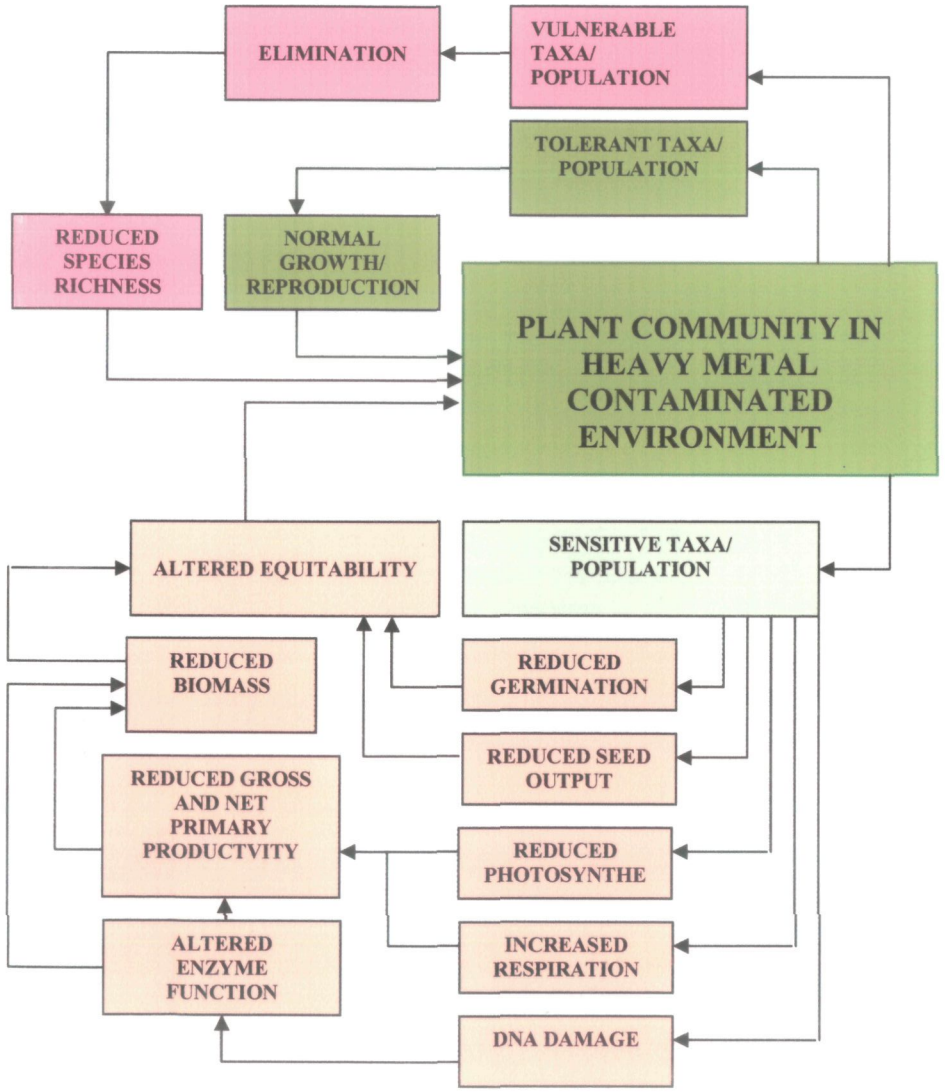

Fig. 5: Hypothetical model for possible mechanism of changes in plant community structure in response to heavy metals.

2. Vulnerable species or subpopulations will either fail to germinate or die before seed setting. In either case such species will be eliminated from the community in present or next generation, thereby bringing about a decline in the number of species or genetic diversity.

3. The sensitive species or subpopulations are likely to respond in diverse ways. Depending on their level of tolerance to heavy metals their productivity may show various degrees of decline commensurate to decrease in photosynthesis. Even if respiration remains unchanged or declines, the productivity will still decrease due to initial low CO2 fixation.

4. Reduced seed output will also change the community structure over a period of time. The species with normal seed output will be an advantageous position, unless their germination is not heavily affected and species with reduced seed output will be represented by lesser number of individuals in next season. Regeneration status of perennial species may also be adversely affected.

PRESENT STUDY

The data, especially those related to dry weight (productivity) and seed output, obtained in present study support this hypothesis.

Fig.6 shows the changes in % contribution of all species to total productivity in communities given acute treatments. In community treated with 0.2 M Chromium nitrate *Chenopodium album* contributed 27.23% while its contribution to control community was 26.33%. In community treated with 0.5 M Chromium nitrate all species showed considerable deviation from the control community. In community treated with 1.0 M Chromium nitrate

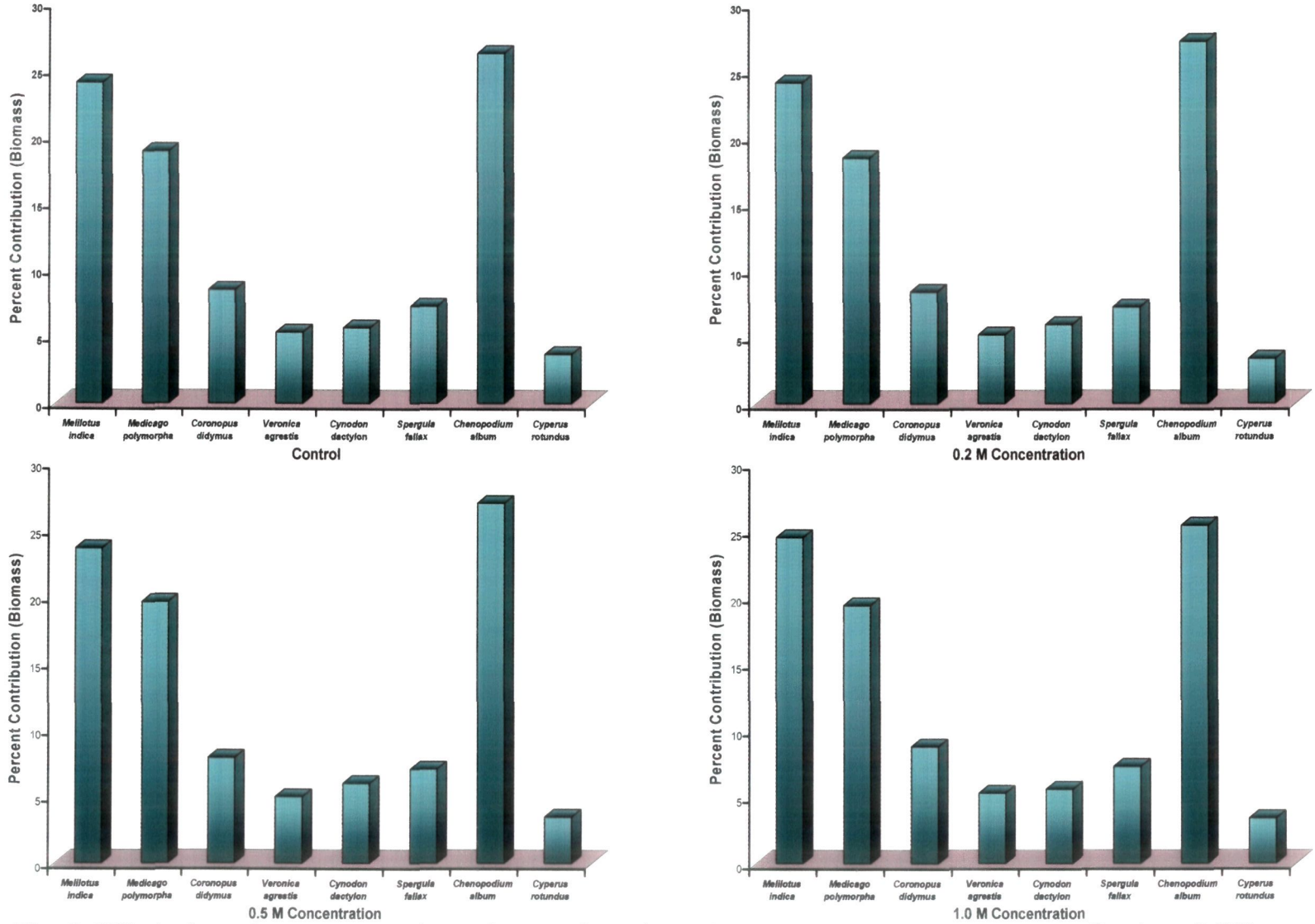

Fig. 6: Effect of varying concentrations of acute chromium nitrate treatment on percent contribution of different plant species to total community productivity.

Medicago polymorpha contributed 20.04% while its contribution to control community was 18. 94%.

Same data were analyzed for % reduction in dry weight of individual species in response to Chromium nitrate treatments (Fig.7). In community treated with 0.2 M Chromium nitrate maximum reduction of 15.75% was shown by *Cyperus rotundus*, followed by *Veronica agrestis* (10.26%), *Medicago polymorpha* (9.43%) and *Coronopus didymus* (8.44%) etc. Treatment with 0.5 M chromium nitrate caused maximum dry weight loss in *Coronopus didymus* (17.27%) followed by *Veronica agrestis* (16.67%), *Cyperus rotundus* (15.75%) and *Spergula fallax* (13.62%) etc. Treatment with 1.0 M chromium nitrate caused maximum loss of 46.19% in *Coronopus didymus*, followed by *Cyperus rotundus* (24.08%), *Chenopodium album* (22.17%), *Cynodon dactylon* and *Veronica agrestis* (19.88% each) and *Spergula fallax* (19.25%) etc. Overall community productivity in response to three treatments was 96.25%, 91% and 82.21% of control respectively.

Chronic treatment with Chromium nitrate also showed more or less same trend, though the severity of response was slightly lower (Fig.8). In community treated with 0.1 M Chromium nitrate, three species *Cynodon dactylon*, *Spergula fallax* and *Chenopodium album* showed slight increase in % contribution to the total productivity in community. All other species showed reduction in % contribution. In response to 0.2 M chromium nitrate treatment again three species *Spergula fallax*, *Chenpodium album* and *Cyperus rotundus* showed slight increase in % contribution, while all other species showed a

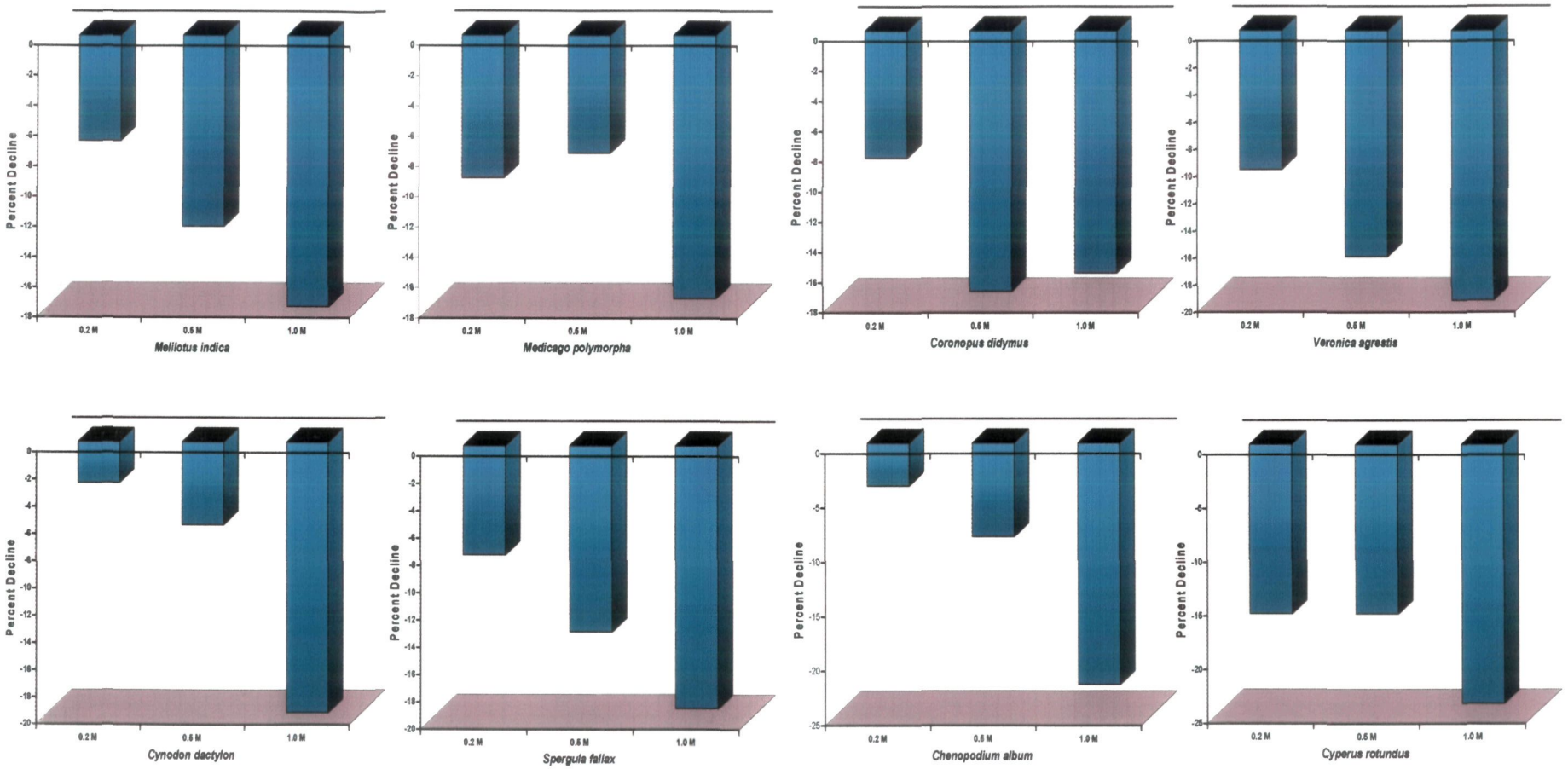

Fig. 7: Effect of varying concentrations of acute chromium nitrate treatment on percent reduction in dry weight of individual plant species.

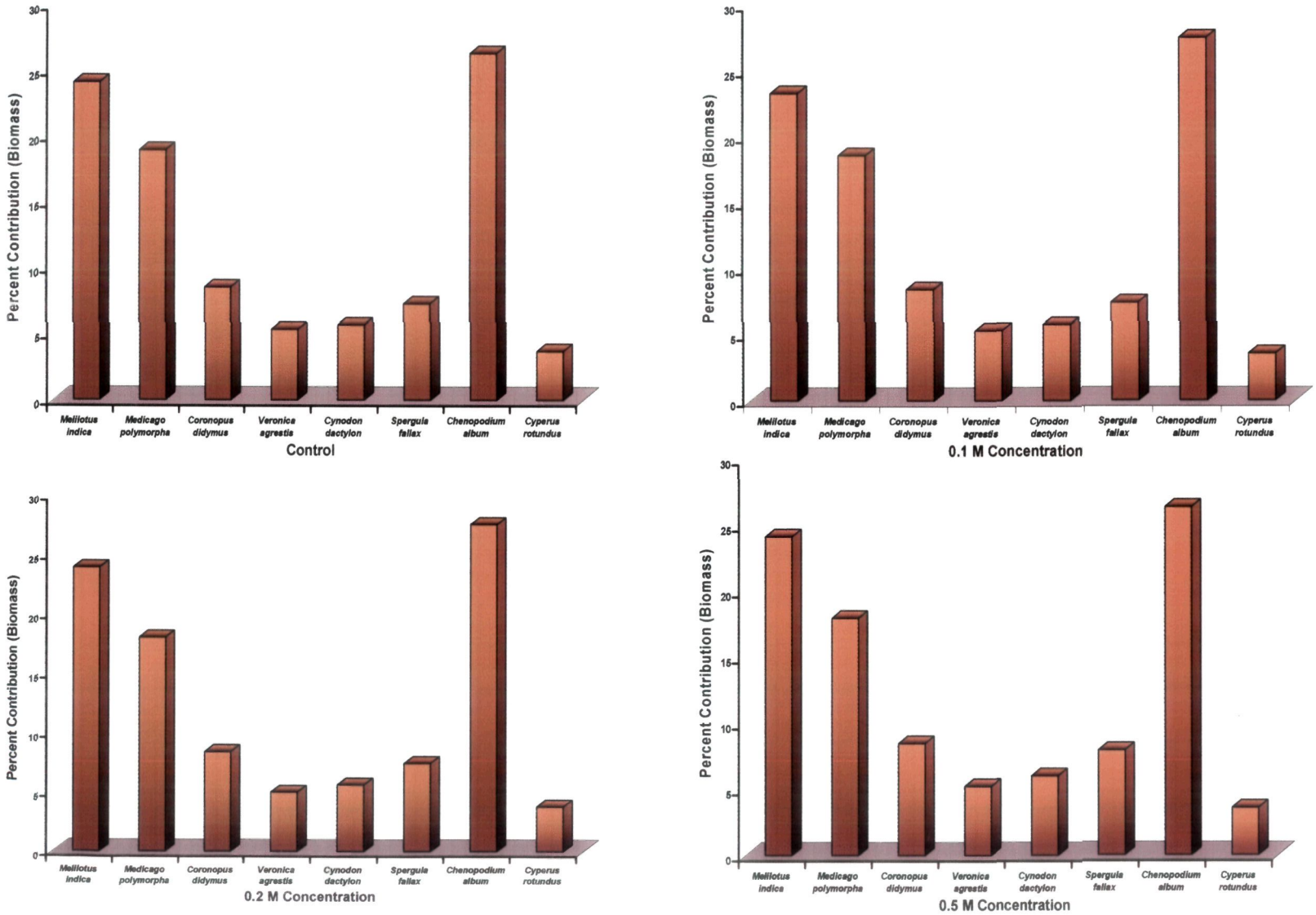

Fig. 8: Effect of varying concentrations of chronic chromium nitrate treatment on percent contribution of different plant species to total community productivity.

decrease in % contribution. In community treated with 0.5 M chromium nitrate *Melilotus indica*, *Cynodon dactylon*, *Spergula fallax* and *Cyperus rotundus* showed slight improvement in % contribution, all remaining species showed decrease in % contribution to the community productivity.

In community treated with 0.1 M chromium nitrate maximum % dry weight reduction was shown by *Cyperus rotundus* (7.41%) followed by *Melilotus indica* (6.8%) and *Coronopus didymus* (4.82%) etc. *Chenopodium album* showed slight improvement of 0.65%. In community treated with 0.2 M chromium nitrate all species showed decrease in biomass. Maximum decrease of 14.75% was shown by *Veronica agrestis*, followed by *Medicago polymorpha* (13.23%), *Coronopus didymus* (10.45%) and *Cynodon dactylon* (9.64%) etc. Treatment with 0.5 M chromium nitrate caused maximum reduction of 19.45% in *Cyperus rotundus* followed by *Veronica agrestis* (19.24%), *Medicago polymorpha* (19.08%) and *Melilotus indica* (17.53%) etc. (Fig.9)

Seed output was calculated as (a) total community seed output (b) seed out put of each species as % of total community seed output at each dose and (c) seed output of individual species in response to various chromium treatments.

Total seed output decreased gradually from lower to higher chromium concentrations (Fig.10). The decline was more pronounced in communities given acute treatment. Fig.11 shows alterations in seed output of six species as per cent of total seed output of community treated with various chromium doses. Clearly, all species do not respond in the same manner. At the highest

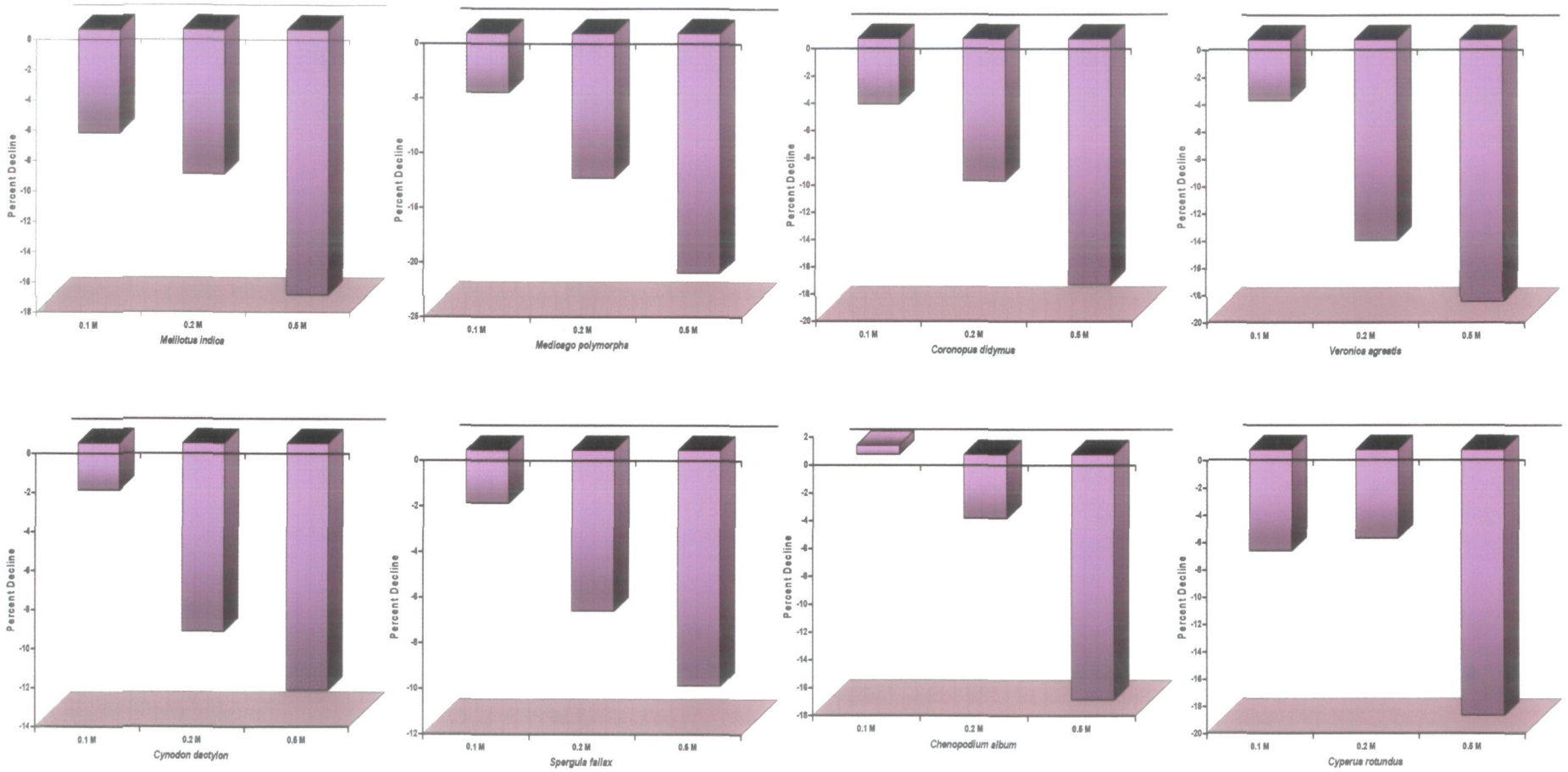

Fig. 9: Effect of varying concentrations of chronic chromium nitrate treatment on percent reduction in dry weight of individual plant species.

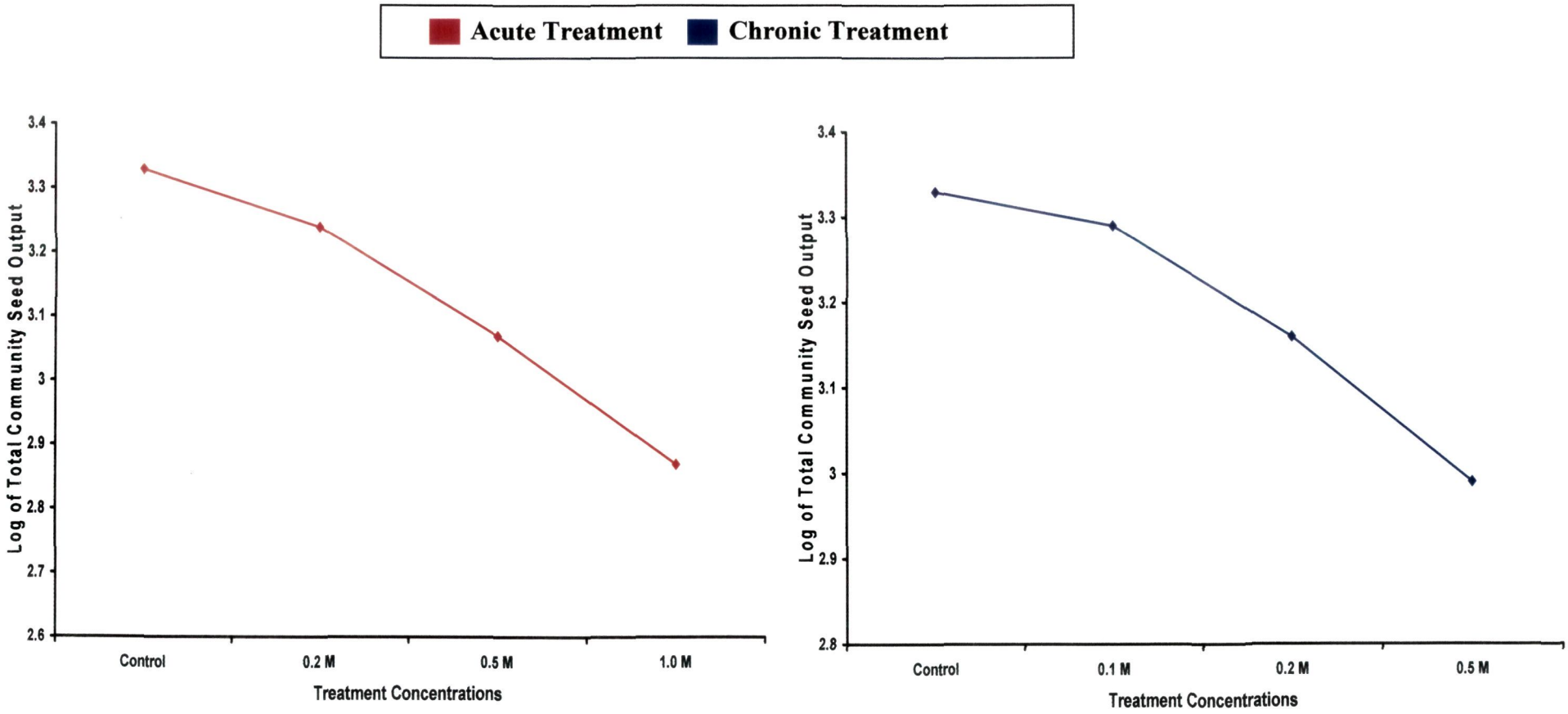

Fig. 10 Effect of varying concentrations of Acute and Chronic chromium nitrate treatments on total community seed output.

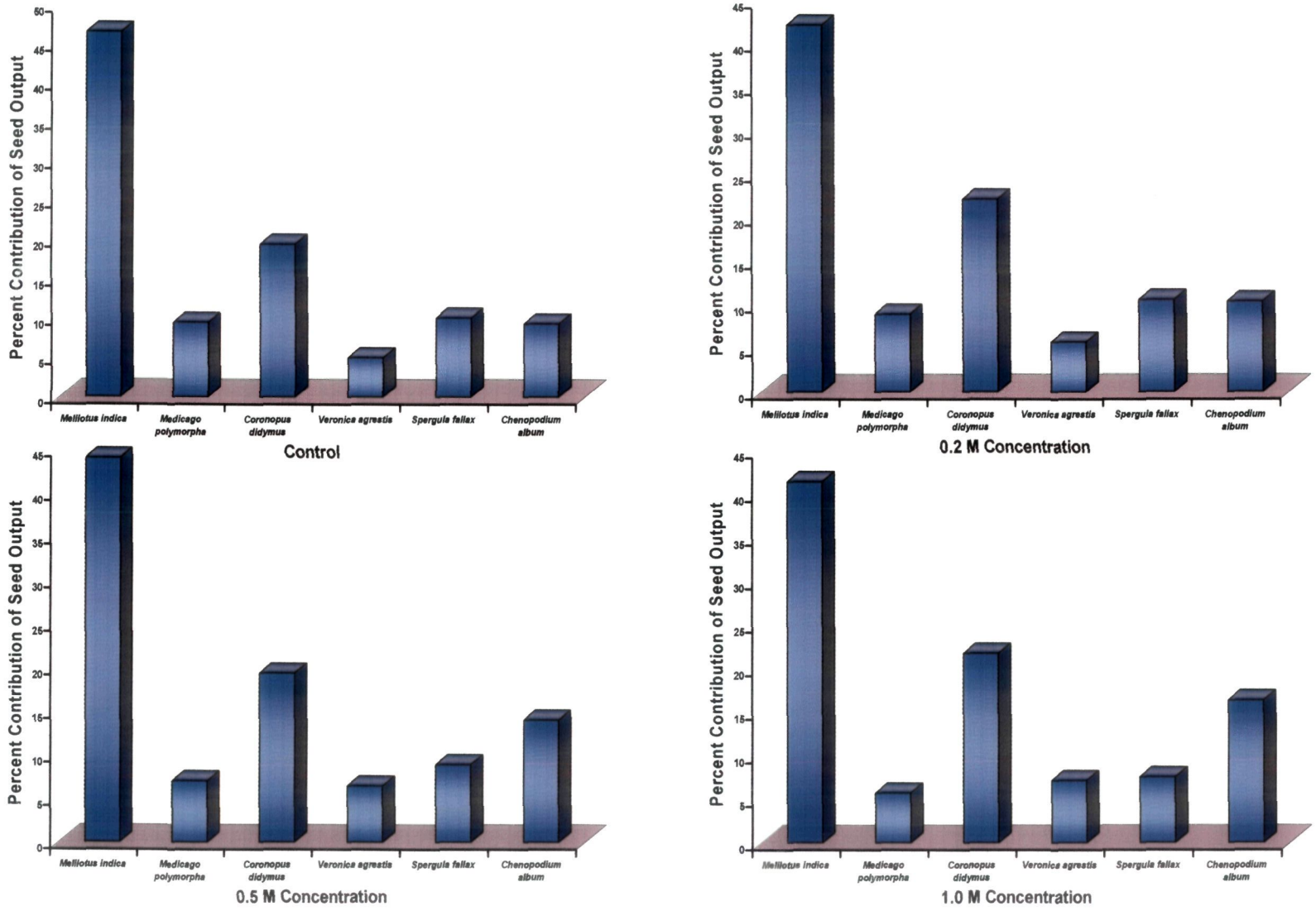

Fig. 11: Effect of varying concentrations of acute chromium nitrate treatment on percent contribution of different plant species to total community seed output.

concentration *Melilotus indica* accounted of 41.5% of total seed output as opposed to 46.5% in control. In response to same treatment the share of *Coronopus didymus* increased by about 2.0%, *Veronica agrestis* by 2.1% and that of *Chenopoium album* by about 7.0%. Thus, *C. album* that ranked fifth in control community ranked third in community treated with 1.0 M chromium. In communities given chronic chromium treatment *Melilotus indica* accounted for greater share of seed output. At highest dose it accounted for 52% of total seed output as compared to 48.5% in control. Total seed output was slightly higher in this treatment in comparison to acute treatment. All other species showed slightly lower seed output as compared to acute treatment.

Still more clear picture emerges from data on % loss of seed output of individual species. In community given acute treatment of 0.2 M chromium, maximum loss (27.88%) was shown by *Melilotus indica* followed by *Medicago polymorpha* (25.1%). Other species showed seed output loss ranging between 10-16%. Treatment with 0.5 M chromium caused maximum loss in *Medicago polymorpha* (59.73%) followed by *Spergula fallax* (52%) and *Melilotus indica* (48.6%) etc. In the community treated with 1.0 M chromium highest seed output loss was shown by *Medicago polymorpha* (79.4%), *Spergula fallax* (76.4%) and *Melilotus indica* (69.5%) etc.

Fig.12 shows alterations in seed output of six species as per cent of total seed output of community treated with chronic chromium doses. Same species in communities given chronic treatment showed seed output loss of relatively lesser degree in comparison to acute treatment. In community treated with

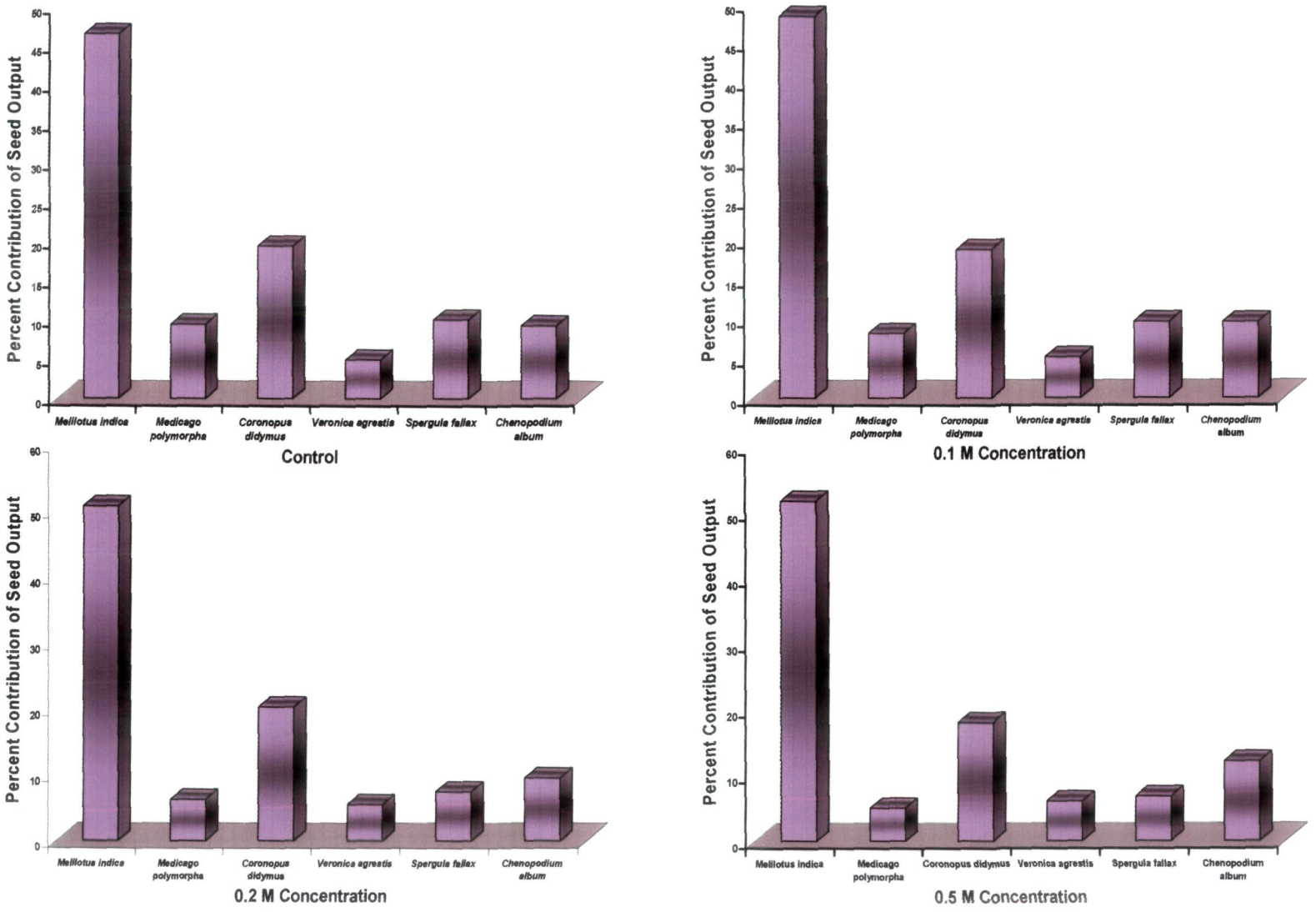

Fig. 12: Effect of varying concentrations of chronic chromium nitrate treatment on percent contribution of different plant species to total community seed output.

0.1% chromium highest loss (23.2%) was shown by *Medicago polymorpha* followed by *Spergula fallax* (14.2%), *Coronopus didymus* (13.75%) etc. In community treated with 0.2 M chromium maximum loss of 31.7% was shown by *Chenopodium album*, followed by *Cornopus didymus* (30.6%), *Melilotus indica* (27.4%) and *Veronica agrestis* (26.85%) etc. Treatment with 0.5 M chromium caused maximum loss in *Medicago polymorpha* (76%) followed by *Spergula fallax* (69.81%), *Coronopus didymus* (58.54%) and *Melilotus indica* (50.2%) etc.

Above discussion may be concluded as follows:

1. No species could be placed in Vulnerable category. Since soil seed bank was not analyzed before study, therefore, the number of species that could have failed to germinate could not be ascertained.

2. The species which showed statistically significant differences in various parameters between control and treated communities belonged to Sensitive category and those which showed statistically non-significant variations were Tolerant.

3. Tolerant or Sensitive nature of species appears to be parameter specific.

4. HM contamination results in reduction of total community productivity.

5. The biomass reduction of individual species is not uniform but varies from species to species.

6. HM contamination also results in altered total community seed output and seed output of individual species. The latter varies from species to species.

FUTURE RESEARCH RECOMMENDATIONS

Present study was in fact a pilot study meant to test certain aspects of the hypothesis formulated to understand the influence of HMs on community structure. As stated above, at least three points of the hypothesis e.g. (a) reduction in total community productivity (a) alteration of individual species biomass and (c) alteration in seed output were corroborated. But the interaction between the biotic and physical environment of a community is a complex matter and the influence exerted by various species upon each other further complicates the problem. Further research should aim to achieve following objectives:

1. Understanding of changes in litter decomposition and consequently nutrient cycling in HM affected habitats.

2. Understanding the population ecology of plant species in HM affected habitats.

3. Understanding the growth and reproduction of multi-species systems in HM affected habitats.

4. Understanding the effect of various heavy metals on community characteristics individually and in combination.

5. Understanding the effect of different heavy metals and their varying doses on same type of community.

Chapter-6
Summary

SUMMARY

Voluminous literature has accumulated over the deleterious affect of heavy metals on living organisms, animals as well as plants. Major portion of studies have focused on individual species and little attention has been paid to important issue of community response to heavy metal contamination. Pollution is one of the major causes of biodiversity loss. Therefore, an understanding of community response to heavy metal contamination will be of immense help in biodiversity conservation.

The primary aim of present study was to develop a hypothesis, on the basis of species responses to heavy metal contamination, to explain and understand the community response to heavy metal contamination (Fig.5) and provide experimental proof for some assumptions of the hypothesis. In fact, a long term study is planned taking into account different communities and heavy metals.

An artificial community consisting of eight local plant species (six dicots and two monocots) was raised in pots and subjected to two heavy metal treatments (acute and chronic) each consisting of three concentrations. Data were collected on root length, shoot length, root dry weight, shoot dry weight, total biomass, mean fruit number per plant and mean seed number per plant. Data were analyzed statistically.

Data obtained supported the classification of pant species into three categories (a) vulnerable (b) sensitive and (c) tolerant. Although no species

could be placed in vulnerable category, but failure of species to germinate or death before seed setting in well documented in literature. All species showed different degree of loss in the parameters studied and therefore belonged to sensitive category. Probably, studies with communities consisting of large number of species would yield species belonging to all three categories.

Altered species and community productivity, measured in terms of total biomass, was clearly observed in this study. More importantly, the productivity loss varied species to species.

Statistically significant changes in mean seed number per plant of all species were observed. Implications of changes in total seed out put for community structure were also discussed.

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